The Influence of Certain Trace Metals on Bacterial Growth and Magnesium Utilization

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SUMMARY

The uptake of Mg^{2+} in Mg^{2+} -limited cultures of the Gram-negative *Escherichia coli* is rapid and is complete some time before the onset of the stationary phase. In similar cultures of the Gram-positive *Bacillus megaterium* and *B. subtilis* F3 growth and Mg^{2+} assimilation cease at the same time and when only part of the available Mg^{2+} has been utilized; thereafter efflux of the cation may occur. In these cultures, as in dilute suspensions of the bacilli in a Mg^{2+} -deficient medium, viability is maintained in a high percentage of the organisms, and growth occurs on the addition of Mg^{2+} , even if this is delayed for 20 hr. The minimum growth-requirement for Mg^{2+} varies for different Gram-positive bacilli and is particularly low for *Bacillus subtilis* var. *niger*. The response of this organism to Mg^{2+} is unaffected by Mn^{2+} . The Mg^{2+} requirements of *B. megaterium* and *B. subtilis* F3, however, are reduced by $25\ \mu M$ Mn^{2+} . Although this concentration of Mn^{2+} is unable to support growth of these bacilli in the complete absence of Mg^{2+} , it appears to stimulate the uptake of the latter cation from dilute solutions. Mn^{2+} also is assimilated during growth by both Gram-positive and Gram-negative bacteria, although less efficiently than Mg^{2+} , and is incorporated into the ribosomes.

INTRODUCTION

In a recent paper Tempest, Dicks & Meers (1967) have reported that the magnesium contents of Mg^{2+} -limited *Bacillus subtilis* var. *niger* and *Aerobacter aerogenes*, when grown in continuous culture at corresponding rates, are not significantly different, and that, at low dilution rates, the uptake of the cation by both of these organisms is almost complete. Tempest *et al.* (1967) conclude from these findings and batch culture experiments with *B. subtilis* var. *niger* and *B. megaterium* that the interpretation of previous observations (Webb, 1966) on the differences in the abilities of certain Gram-positive bacilli and Gram-negative bacteria in batch culture to concentrate Mg^{2+} from simple chemically defined media is incorrect. The authors suggest that any variation in the response of these organisms to low concentrations of Mg^{2+} must be due to physiological factors other than differences in assimilation, and in this connexion infer the importance of a differential effect of Mg^{2+} deficiency on the death rates of Gram-positive and Gram-negative organisms in aqueous environments. This hypothesis, which presumably would require the more efficient retention of Mg^{2+} by the Gram-negative bacteria, is supported by the difference that has been observed in the relative binding affinities of *A. aerogenes* and *B. subtilis* var. *niger* for Mg^{2+} (Tempest *et al.* 1967).

Several other explanations for the discrepancies between the work of Webb (1966)

and of Tempest *et al.* (1967), however, are possible. First, the results that are described in the two papers refer to cultures in different phases of growth, i.e. the stationary and logarithmic phases respectively. The cellular content of Mg^{2+} is related to that of RNA (Dicks & Tempest, 1966; Tempest, Dicks & Hunter, 1966), which is known to decrease at the end of exponential growth (e.g. Malmgren & Hedén, 1947). It is possible therefore that the content per organism of bound Mg^{2+} is greater in exponentially growing than in stationary populations, and that in the Gram-positive bacilli this excess cation is returned to the medium at the end of the logarithmic phase.

Secondly, the Gram-positive bacilli differ amongst themselves with regard to the Mg^{2+} concentration that is required to initiate growth (Webb, 1966). It is possible, therefore, that *Bacillus subtilis* var. *niger* may assimilate Mg^{2+} more efficiently and thus exhibit a lower requirement for the cation than the organisms that have been studied hitherto.

Thirdly, the growth medium of Tempest *et al.* (1967) contains 'trace amounts' of other bivalent cations (Cu^{2+} , Mn^{2+} , Zn^{2+}) and of the molybdate anion. Any of these ions, which are not included in Webb's (1966) medium, may influence the utilization of Mg^{2+} .

The present paper is concerned with an investigation of these possibilities. It is shown that, in contrast to the Gram-negative *Escherichia coli*, efflux of cellular Mg^{2+} does occur when cultures of the Gram-positive bacilli enter the stationary phase, but in Webb's (1966) Mg^{2+} -limited medium the assimilation of the cation is incomplete even during the logarithmic growth phase. The Mg^{2+} requirements of these organisms, however, are reduced considerably by the presence of Mn^{2+} at the concentration ($25 \mu M$) that is included in the medium of Tempest *et al.* (1967). Under these conditions growth occurs at low concentrations of Mg^{2+} and the uptake of this cation is proportionately greater. In the absence of Mn^{2+} and other trace metals, the response of *Bacillus subtilis* var. *niger* to Mg^{2+} is similar to that of other Gram-positive bacilli, although the concentration of the cation that is necessary to initiate growth is much lower.

METHODS

Organisms. The sources and conditions of maintenance of *Bacillus megaterium* (KM), *B. subtilis* (F 3), *B. mesentericus* and *Escherichia coli* have been given previously (Webb, 1966). *B. subtilis* var. *niger* was obtained from Dr D. W. Tempest and was maintained by monthly subculture on Evans peptone-agar slopes containing 0.2 % glucose.

Growth conditions. Cultures were grown in shaken flasks at 37° in either P medium (Webb, 1966) or T medium (Tempest *et al.* 1967). These solutions were supplemented with Mg^{2+} and other ions as stated in the text and were sterilized by filtration through sintered glass filters. Inocula for the experimental series were taken from organisms that had been subcultured at least three times in the appropriate defined medium. Growth was measured turbidimetrically as described previously (Webb, 1966).

Viable counts were made by plating on to Evans peptone (2 %, w/v)-glucose (0.2 %, w/v)-agar (2 %, w/v).

Isolation of ribosomes from cells grown in the presence of $^{54}Mn^{2+}$. Cultures were grown in P medium (500 ml.) supplemented with $^{54}Mn^{2+}$ ($10 \mu C$; The Radiochemical Centre, Amersham, Bucks.) and Mg^{2+} as indicated in Table 2. After being harvested and washed 3 times with solution L (0.01 M-tris buffer, pH 7.4, 0.06 M-KCl and

0.014 M- $(CH_3COO)_2Mg$, the organisms were resuspended in the same solution (15 ml.) and treated at 0° with ultrasonic vibrations in an M.S.E. machine (4×30 sec. at 1.5 A with 30 sec. intervals of rest). This method, which yielded ribosomal preparations that were comparable with those obtained from organisms that were crushed in the French press, was used in preference to the more conventional procedures for the disintegration of the labelled organisms. The homogenates were treated with DNase (1 $\mu g./ml.$; Sigma Chemical Co., London; RNase-free) for 5 min. at room temperature and then centrifuged at 4° for 10 min. at 18,000 g. The supernatant fractions were centrifuged for 70–90 min. at 114,000 g (Spinco Model L centrifuge, no. 40 rotor) and 0°. The crude ribosomal pellets were dissolved in mixture L, the solutions centrifuged for 5 min. at 12,000 g to remove insoluble and aggregated material, and then diluted as necessary to contain 0.9–1.0 mg. RNA/ml. Portions (0.2 ml.) of these solutions were layered on to continuous gradients of 10%–25% (w/v) sucrose in mixture L, and centrifuged for 100 min. at 125,000 g and 4° in the no. 39 rotor of the Spinco ultracentrifuge. Three gradients were run with each crude preparation. After centrifugation the bottom of each tube was pierced with a no. 16 hypodermic needle, and a series of 20-drop fractions was collected. These fractions were diluted with solution L (1.0 ml.) and the main ribosomal components located by measurement of the E_{260} values. The appropriate fractions from each series were combined and the ribosomes were recovered by centrifugation for 90 min. at 114,000 g. The particle dry weight was calculated from the decrease in the E_{260} value on centrifugation of each solution according to Imsande & Caston (1966).

Measurement of radioactivity. The ribosomal or cell pellets were dissolved in 98% (w/v) formic acid (0.5 ml.) at 60° before assay. Radioactivity was measured as described by Daniel, Dingle, Webb & Heath (1963).

Analytical methods. Mg^{2+} and Mn^{2+} were determined in culture supernatants by atomic absorption, the former by direct analysis in the presence of La^{3+} (Webb, 1966) and the latter by scale expansion in conjunction with the Perkin Elmer Recorder Readout accessory.

RESULTS

*Utilization of Mg^{2+} in Mg^{2+} -limited cultures of *Escherichia coli*, *Bacillus megaterium* and *B. subtilis* F3*

Variation of the Mg^{2+} content of P medium over the range 0.25–2.0 $\mu g\ Mg^{2+}/ml.$ influenced both the rate and duration of the logarithmic growth phase of *Escherichia coli*, but had little effect on the length of the initial lag (Table 1). In Mg^{2+} -limited cultures of this organism the uptake of Mg^{2+} occurred rapidly, and the concentration of the cation in the medium was reduced to zero, usually within $2\frac{1}{2}$ –3 hr of the end of the lag phase (Fig. 1). After the complete utilization of the available Mg^{2+} , which correlated approximately with the transition from logarithmic growth to the phase of decreasing multiplication rate, the cell density increased by at least 33% in the absence of the exogenous cation. In cultures that contained initially 0.2 and 0.5 $\mu g.\ Mg^{2+}/ml.$ the cell densities reached maximum values and then decreased by about 32% and 21% respectively between 8 and 20 hr. Mg^{2+} ions were not liberated from the cells during this period. At higher levels of Mg^{2+} (1.5–4.0 $\mu g.\ Mg^{2+}/ml.$) some slight efflux of the cation occurred between 6 and 8 hr (Table 1).

In agreement with previous findings (Webb, 1966), the growth of *Bacillus mega-*

terium and *B. subtilis* F 3 in P medium was limited to a greater extent by low concentrations of Mg^{2+} than was that of *Escherichia coli* (Fig. 1). Throughout the period of incubation of cultures of the Gram-positive bacilli the utilization of Mg^{2+} from media with initial concentrations of 0.5, 0.9 and 1.2 $\mu g.$ $Mg^{2+}/ml.$ was not quantitative. In

Fig. 1. Growth (●—●) and magnesium utilization (○ --- ○) in cultures of *Escherichia coli* with 0.5 $\mu g.$ $Mg^{2+}/ml.$, and in cultures of *Bacillus megaterium* with 0.9 $\mu g.$ $Mg^{2+}/ml.$ in P medium.

Table 1. *Magnesium utilization in Mg^{2+} -limited cultures of *Escherichia coli**

The experimental cultures were grown with shaking in P medium (15 ml.) supplemented with different amounts of Mg^{2+} in conical flasks (50 ml.) that were fitted with side arms for turbidimetric measurements. At the intervals shown, portions (3.0 ml.) of the cultures were removed aseptically, centrifuged and the supernatant fractions analysed for Mg^{2+} . The inocula (0.3 ml.) were taken from a 16 hr culture in P medium with 2 $\mu g.$ $Mg^{2+}/ml.$

Time (hr) at 37°	Mg ²⁺ concentration (μg./ml.)					
	μg. dry wt organisms/ml.					
	0.2	0.5	1.0	1.5	2.0	4.0
1.5	19	23	26	26	24	32
2.0	40	33	33	37	36	41
3.0	85	94	79	78	90	90
3.75	122	154	154	156	159	163
4.0	137	188	204	206	214	217
5.0	169	263	379	409	430	440
6.0	180	302	459	539	595	714
7.0	199	344	514	616	775	794
8.0	197	359	533	735	760	950
	Residual Mg ²⁺ (μg./ml.)					
2.0	0.18	0.50	0.87	1.32	1.79	3.42
4.0	0.04	0.03	0.19	0.66	1.02	2.66
6.0	0.02	0.01	0.03	0.03	0.01	0.03
8.0	0.00	0.00	0.00	0.01	0.04	0.10

each culture, the cation content of the medium decreased to a minimum at the onset of the stationary phase and then increased. At the low medium concentrations of Mg^{2+} the liberation of the cation coincided with lysis, as shown by the decrease in turbidity of the cultures after 3 hr. At the higher levels of Mg^{2+} , however, turbidity remained essentially constant between 8 and 20 hr.

Mg²⁺ requirements of Bacillus subtilis var. niger

In cultures of *Bacillus subtilis* var. *niger* in P medium the lag in the growth response to increasing concentrations of Mg^{2+} was much shorter than with other Gram-positive bacilli that have been studied previously in batch culture (Webb, 1966). Growth began at about $0.15\text{--}0.2\text{ }\mu\text{g. Mg}^{2+}/\text{ml.}$, and then increased rapidly with the concentration of the cation, whilst in cultures that contained low levels of Mg^{2+} the utilization of the cation was almost complete.

Fig. 2. Growth (---), and Mg^{2+} utilization (—) as functions of Mg^{2+} concentration in cultures of *Bacillus subtilis* F 3, in T medium (O) and P medium (●).

Influence of other ions on the utilization of Mg^{2+} by Gram-positive bacilli. In the medium of Tempest *et al.* (1967), both the growth response to Mg^{2+} and the utilization of the cation by *Bacillus subtilis* F 3, *B. megaterium* and *B. mesentericus* were different from those in P medium. Thus in T medium the growth lag at low levels of Mg^{2+} was eliminated and the utilization of the cation at concentrations below $0.1\text{ }\mu\text{g. Mg}^{2+}/\text{ml.}$ was almost complete (Fig. 2). Under these conditions there was little difference, for example, in the response of *B. subtilis* F 3 (Fig. 2) and of *B. subtilis* var. *niger*.

As the major components of P and T media were similar, these observations indicated that the Mg^{2+} -requirements of at least certain Gram-positive bacilli were modified by the additional trace metals (Mn^{2+} , Cu^{2+} , Zn^{2+} and MoO_4^{2-}) of T medium. In P medium the Mg^{2+} requirements for growth of *Bacillus subtilis* F 3 and *B. megaterium* were reduced greatly by Mn^{2+} , but were increased by $Cu^{2+}Zn_4^{2+}$ and MoO_4^{2-} when these ions were added separately in the amounts present in T medium (Fig. 3). Although Mn^{2+} ($25\text{ }\mu\text{M}$) alone was unable to support the growth of these organisms, it stimulated the uptake of Mg^{2+} when this ion was present in low concentration. With

B. subtilis var. *niger* the utilization of Mg^{2+} was increased slightly by Mn^{2+} , and decreased by Cu^{2+} , Zn^{2+} and MoO_4^{2-} , although none of these ions had any significant effect on the growth response of this organism to increasing concentrations of Mg^{2+} . The latter finding was confirmed by a study of the response of *B. subtilis* var. *niger* to Mg^{2+} in T medium from which the various trace metal components were omitted separately. When Mn^{2+} , Cu^{2+} and MoO_4^{2-} were omitted together, however, growth did not occur at Mg^{2+} concentrations lower than $0.15 \mu g./ml.$, and at $2 \mu g./ml.$ the cell

Fig. 3. Influence of other ions on growth (upper figures) and Mg^{2+} utilization (lower figures) in Mg^{2+} -limited cultures of *Bacillus megaterium* (a, c) and *Bacillus subtilis* F3 (b, d). Additions to P medium were as follows: none (▲), $25 \mu M$ - Mn^{2+} (●), $5 \mu M$ - MoO_4^{2-} (■), $10 \mu M$ - Zn^{2+} (●), $5 \mu M$ - Cu^{2+} (○).

density in fully grown cultures was 25 % less than in the complete medium. In some experiments with this organism in the absence of Cu^{2+} , Zn^{2+} and MoO_4^{2-} , Mn^{2+} ($25 \mu M$) antagonized the uptake of Mg^{2+} at concentrations of less than $0.5 \mu g./ml.$ and, at the lowest levels of Mg^{2+} , caused the liberation of this cation from the cells of the nocolum. This antagonism, however, was not consistently reproducible. At higher concentrations (1.0 – $2.0 \mu g./ml.$) of Mg^{2+} , Mn^{2+} acted synergistically, the increase in dry wt organisms/ml. under these conditions being 95–98 % of that in corresponding cultures in the complete medium, and the uptake of Mg^{2+} about 35 % greater.

Utilization of Mn^{2+} by Escherichia coli, Bacillus megaterium and B. subtilis var. *niger*. Analysis of cell-free supernatant fractions from the above experiments established that, irrespective of the Mg^{2+} concentration, only a small fraction of the available Mn^{2+} was assimilated by each of the three organisms. As the decrease in the content of this cation in the media was too low to be determined accurately by atomic absorption, uptake was measured with $^{54}Mn^{2+}$ as tracer.

In *Bacillus megaterium* and *B. subtilis* var. *niger*, as in *Escherichia coli*, Mn^{2+} was taken up during growth and incorporated into the ribosomes (Table 2). It is probable that in the three organisms the Mn^{2+} contents of the native, intracellular ribosomes were higher than those found for the isolated particles, since these were prepared in the presence of a relatively high concentration (14 mM) of Mg^{2+} to prevent dissociation to the 50 S and 30 S sub-units. Although the binding affinity of bacterial (*E. coli*) ribosomes for Mn^{2+} is about 3 times that of Mg^{2+} (Sheard *et al.* 1967) it is to be expected that some displacement of the former cation by the latter would occur under the conditions of the present experiments. In culture, Mg^{2+} antagonized the uptake of Mn^{2+} . Thus, as shown in Table 2, a 10-fold increase in the Mg^{2+} concentration of the medium depressed the Mn^{2+} content of the whole cells and isolated ribosomes of *E. coli* by 87 % and 80 % respectively. In this connexion it is interesting that the Mn^{2+}

Table 2. Incorporation of $^{54}Mn^{2+}$ by *Escherichia coli*, *Bacillus megaterium* and *Bacillus subtilis* var. *niger*

The cultures were grown with aeration at 37° in P medium (500 ml.) supplemented with 12.5 μ moles Mn^{2+} (10 μ C $^{54}Mn^{2+}$) and Mg^{2+} as shown, from inocula (5 ml.) that contained medium concentrations of Mg^{2+} of 1 μ g./ml. (*E. coli*), 2 μ g./ml. (*B. subtilis* var. *niger*) and 5 μ g./ml. (*B. megaterium*). Growth was followed turbidimetrically to enable the two cultures of *E. coli* and of the Gram-positive bacilli to be harvested in the logarithmic phase at approximately the same dry wt organisms/ml. The procedures for the isolation of ribosomes and the determination of Mn^{2+} were as described in the Materials and Methods section.

Organism	Mg^{2+} content of culture medium (μ g./ml.)	Yield (mg. dry wt organisms)	Total Mn^{2+} incorporated by cells (μ moles)	Mn^{2+} content (m μ -moles/mg. dry wt)	
				Cells	Ribosomes
<i>E. coli</i>	0.5	94.6	0.123	1.30	1.32
<i>E. coli</i>	5.0	91.7	0.016	0.17	0.27
<i>B. subtilis</i> var. <i>niger</i>	1.0	134.7	0.122	0.81	1.04
<i>B. megaterium</i>	2.0	148.0	0.264	1.78	1.81

contents of *B. megaterium* cells and ribosomes were approximately double those of *B. subtilis* var. *niger*, although the Mg^{2+} concentration in the culture of the former was twice that of the latter. It seems therefore that *B. megaterium* may have a lower affinity for Mg^{2+} than *B. subtilis* var. *niger*, but a higher affinity for Mn^{2+} .

Loss of viability in Gram-positive bacilli in the absence of Mg^{2+}

Although *Bacillus megaterium*, *B. subtilis* F 3 and *B. subtilis* var. *niger* failed to grow in either P or T medium in the absence of Mg^{2+} , viability was maintained. With both *B. subtilis* F 3 and *B. megaterium*, the viable count fell by about 45 % during 20 hr at 37°, a 25 % decrease being observed within the first 3.5 hr of incubation. The death-rates of these organisms in the Mg^{2+} -deficient P medium were little affected by Mn^{2+} , Cu^{2+} and MoO_4^{2-} , but were increased by Zn^{2+} (10 μ M), in some experiments by as much as 90–95 % in 6 hr. Even in the presence of Zn^{2+} some cells survived for 20 hr and were capable of growth when the medium was supplemented with Mg^{2+} (10 μ g./ml.).

In cultures of *Bacillus subtilis* F 3 in P medium with 0.5 μ g. Mg^{2+} /ml., as in those of *B. megaterium*, there was no stable stationary state, but a decrease in cell density after 6 hr (Fig. 4). This decrease in density was exaggerated by the turbidimetric measurements since once the growth maximum was attained, the cells tended to

agglutinate. Agglutination was correlated with a change in morphology from Gram-positive rods to Gram-negative filaments of uneven thickness, the majority of which were swollen at one or both ends. Sporulation did not occur. With most Gram-positive bacilli, the formation of filaments in response to Mg^{2+} deficiency occurs in complex media, but not in simple nutrient solutions unless these are supplemented with amino acids (Webb, 1951*b*). This atypical behaviour of *B. subtilis* F 3, however, has been described previously by Grunau (1958).

Fig. 4. Response of Mg^{2+} -limited cultures of *Bacillus subtilis* F 3 to Mg^{2+} . The parent culture (○—○) was grown with shaking in P medium with limited Mg^{2+} ($0.5 \mu g./ml.$) at 37° under the conditions described in Table 1. Portions of this culture were supplemented with additional Mg^{2+} ($10 \mu g./ml.$) at 0 hr (○---○), 4.75 hr (●---●), 5.75 hr (▲---▲), 7 hr (■---■), and 7.75 hr (▼---▼).

On the addition of Mg^{2+} ($10 \mu g./ml.$) to a Mg^{2+} -limited culture of *Bacillus subtilis* F 3 growth recommenced with little or no lag with the production of a new population of morphologically normal, Gram-positive cells. An interesting and unexpected feature of these results (Fig. 4) was that the rate of multiplication in the second growth phase increased the later the Mg^{2+} was added, and was greatest during the period of the decrease in turbidity of the parent culture. It appears therefore that the Mg^{2+} requirements of cells which survive during the period of degeneration in Mg^{2+} -deficient cultures of *B. subtilis* F 3, and which resume growth on the addition of Mg^{2+} , are reduced by the presence of products from other autolysed cells. Certain amino acids, for example, are known to reduce the Mg^{2+} requirements of various Gram-positive bacilli in simple media (Webb, 1951*b*, 1966).

Effect of other ions on the leakage of Mg^{2+} from Gram-positive bacilli

Previously (Webb, 1966) it was reported that when Gram-positive bacilli are transferred to a Mg^{2+} -deficient medium leakage of Mg^{2+} occurs progressively with time and often precedes a decrease in cell density. A number of observations suggest that a bivalent cation, usually considered to be either Mg^{2+} or Ca^{2+} , is necessary to maintain

the integrity of the bacterial cell wall, cell membrane or permeability barriers (e.g. Strange, 1964; Gray & Wilkinson, 1965; Hamilton-Miller, 1966; Goldman, 1966). The trace amounts of Cu^{2+} and Mn^{2+} in the medium of Tempest *et al.* (1967) also appeared to stabilize Gram-positive cells and, in the absence of exogenous Mg^{2+} , prevented both the leakage of this cation and the decrease in cell density. The loss of Mg^{2+} which occurred, for example, when cells from the exponential phase of a culture of *Bacillus subtilis* in P medium with 2 μg . Mg^{2+}/ml . were transferred to fresh medium without Mg^{2+} was prevented completely by Cu^{2+} (5 μM) and reduced by 85 % in the presence of Mn^{2+} (25 μM).

DISCUSSION

The present results confirm and extend those reported previously (Webb, 1966) on the difference in the abilities of certain Gram-positive and Gram-negative bacteria in batch culture to concentrate Mg^{2+} from a simple chemically defined medium. In Mg^{2+} -limited cultures of the Gram-negative *Escherichia coli* for example, the utilization of the cation is rapid and is complete some time before the stationary phase is reached. In contrast, in similar cultures of the Gram-positive *Bacillus megaterium* and *B. subtilis* F 3 only part of the available Mg^{2+} is utilized; growth and Mg^{2+} assimilation cease at the same time, and thereafter efflux of the cation may occur. This efflux of Mg^{2+} may be either accompanied or followed by some cell lysis. In such stationary cultures of the Gram-positive bacilli, as in dilute cell suspensions in Mg^{2+} -deficient media, some cells remain viable and are able to grow on the addition of Mg^{2+} , even if this is delayed for 20 hr. Thus contrary to the suggestion of Tempest *et al.* (1967), loss of viability of the Gram-positive bacilli cannot account for the differences in the growth response of these and Gram-negative bacteria to low concentrations of Mg^{2+} .

The concentration of Mg^{2+} that is necessary to initiate growth of various Gram-positive bacilli varies with the species (Webb, 1949, 1966), and appears to be extremely small for *Bacillus subtilis* var. *niger*, the organism that has been used in most of the comparative studies of Tempest *et al.* (1967). This strain of *B. subtilis* is able to grow in the P medium, and to assimilate Mg^{2+} , at concentrations of the cation that are inadequate for a number of other bacilli.

In cultures of certain of the Gram-positive bacilli the Mg^{2+} requirements for growth are reduced by Mn^{2+} (25 μM). The synergistic action of Mn^{2+} is in agreement with previous observations (Webb, 1951 *a*) on the partial ability of this cation to substitute for Mg^{2+} in the nutrition of these organisms. The present results, however, show that in the complete absence of Mg^{2+} this limited concentration of Mn^{2+} is unable to support growth of either the Gram-negative *Escherichia coli* or a number of Gram-positive bacilli, and suggest that either there is some fundamental reaction that has absolute specificity for the former cation, or the transport of Mn^{2+} is energy-dependent and is activated by Mg^{2+} . Assimilation of Mn^{2+} is much less efficient than is that of Mg^{2+} , and, at least in *E. coli*, is decreased by increased concentrations of the latter cation. It seems therefore that the relationship between the utilization of these two ions may be synergistic or antagonistic according to their relative concentrations.

It is significant that in the presence of low levels of Mg^{2+} , Mn^{2+} is taken up by bacterial cells and incorporated into the ribosomes (Table 2), since, chemically, the inhibition of protein synthesis is the main result of Mg^{2+} deficiency, particularly in Gram-positive bacilli (Webb, 1953). Kennell & Kotoulas (1967) have shown that

Mn^{2+} partially protects the ribosomes of *Aerobacter aerogenes* against degradation due to Mg^{2+} deficiency, whilst the ability of Mn^{2+} to exchange rapidly with the bound Mg^{2+} of isolated ribosomes from *E. coli* has been described by Sheard *et al.* (1967). Earlier, Tissières, Schlessinger & Gros (1960) reported that Mn^{2+} was 50 % as efficient as Mg^{2+} in the activation of protein synthesis by *Escherichia coli* ribosomes. It seems therefore that incorporation of Mn^{2+} into the ribosomes of Mg^{2+} deficient bacteria would maintain the structure and function of the particles, and also liberate some bound Mg^{2+} , which would thus become available for other processes.

The reduced Mg^{2+} requirements of a number of Gram-positive bacilli in the medium of Tempest *et al.* (1967) can be explained by the presence of Mn^{2+} . Individually, the other trace metal components of this medium have little effect on either growth or Mg^{2+} utilization, although under certain conditions Cu^{2+} may stabilize the cells and prevent both the efflux of Mg^{2+} and partial lysis that are liable to occur in the absence of the latter cation. The discrepancies between the results of Tempest *et al.* (1967) and of Webb (1966) thus seem to be due to the use by the former authors of (a) *Bacillus subtilis* var. *niger*, a Gram-positive bacillus that is atypical in its Mg^{2+} requirements, and (b) a medium that contains Mn^{2+} , which can substitute, at least in part, for Mg^{2+} in the nutrition of both Gram-positive and Gram-negative bacteria.

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