The Utilization of Magnesium by Certain Gram-Positive and Gram-negative Bacteria

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SUMMARY

Several Gram-positive bacilli and Gram-negative bacteria were found to differ markedly in their ability to concentrate Mg^{2+} from simple chemically defined media. In cultures of the Gram-negative bacteria the uptake of Mg^{2+} was quantitative over the range where growth was limited by the concentration of Mg^{2+} . The Gram-positive bacilli, however, were unable to utilize Mg^{2+} when the external concentration was low, and at higher concentrations sufficient to support about half maximal growth, assimilated less than 50% of the available Mg^{2+} . Both Gram-negative and Gram-positive organisms initially liberated Mg^{2+} when suspended in Mg^{2+} -free media, the Mg^{2+} being re-utilized rapidly by the Gram-negative organisms. In suspensions of *Aerobacter aerogenes* the liberation of Mg^{2+} was independent of temperature and the re-utilization of Mg^{2+} was dependent on temperature.

INTRODUCTION

Although the magnesium requirements for maximal growth in simple chemically defined media are some ten times greater for Gram-positive bacteria than for Gram-negative bacteria (Webb, 1949*a*) the magnesium contents of these organisms do not differ greatly (Webb, 1949*b*; Rouf, 1964), and it is probable that the intracellular concentrations of free cation are similar. De Ley's (1964) observations, for example, on the unity of ribosomes from widely divergent Gram-positive and Gram-negative bacteria, coupled with the known dependence of the structure of polysomes from mammalian, plant and microbial cells on similar concentrations of Mg^{2+} , indicate that the intracellular content of the free cation probably must be maintained within certain critical limits. The present study concerns the utilization of magnesium in bacteria growing in simple media. It is shown that the previously observed variations in the requirements of the Gram-positive and Gram-negative species are due to differences in assimilatory efficiency.

METHODS

Organisms. *Bacillus megaterium* (strain KM) was obtained from Dr K. McQuillen (Department of Biochemistry, University of Cambridge), *Salmonella typhimurium* (strain ara 9) from Dr H. E. Umbarger (Long Island Biological Association, Cold Spring Harbor, New York), and *Escherichia coli* (strain B) from the Department of Molecular Biology, University of Cambridge. Other organisms, the sources of which were given previously (Webb, 1949*b*), were available from the Strangeways

Laboratory collection. These were maintained on peptone (British Drug Houses Ltd.) agar (Oxoid Ltd.) slopes, and were transferred at about monthly intervals. Before being used in the Mg^{2+} uptake experiments the organisms were subcultured at least twice in the chemically defined medium with adequate magnesium (10 $\mu g./ml.$) and then once at a low concentration of Mg^{2+} .

Analytical methods. Magnesium was determined by atomic absorption in a Perkin-Elmer Model 303 Spectrophotometer. Samples of medium for analysis were treated with the appropriate amount of a solution of La^{3+} in HCl and diluted with glass-distilled water to contain 0.2–2.0 $\mu g. Mg^{2+}/ml.$, 0.1 % (w/v) La^{3+} and 0.06 N-HCl. Since under these conditions, interference due to the high contents of phosphate and other salts was not suppressed completely, additional standards were prepared from known amounts of a previously analysed aqueous solution of $MgCl_2$ and appropriate dilutions of the 'magnesium-free' chemically defined media. Extrapolation of these curves to zero absorbance also enabled the magnesium content of the basal media to be determined accurately.

The stock solution of La^{3+} (5 %, w/v) in 3 N-HCl (AnalaR; British Drug Houses Ltd.) was prepared from lanthanum acetate (Hopkins and Williams Ltd.). All other commercially available soluble lanthanum salts and the oxide which were examined were highly contaminated with magnesium, and gave excessively high reagent blanks.

For the determination of cellular magnesium content, portions of the bacterial suspensions (of known dry weight) were mixed with 25 % (w/v) trichloroacetic acid (1.0 ml.) and diluted with glass-distilled water to 5.0 ml. The bacteria were dispersed with a Whirlimixer (Scientific Industries International Inc. (U.K.) Ltd.) and the suspensions left overnight at room temperature before being centrifuged. Portions of the supernatant solutions after centrifugation were supplemented with La^{3+} and HCl as above, and analysed by atomic absorption. Parallel determinations on H_2SO_4 -digests of suspensions of *Aerobacter aerogenes* and *Escherichia coli* were made to confirm that quantitative liberation of Mg^{2+} was obtained by the TCA-extraction procedure.

Culture media and growth conditions. All glassware was cleaned by the methods previously described (Webb, 1949a).

The compositions (g./l.) of the chemically defined media made up in glass-distilled water, were as follows:

Medium M: $(NH_4)_2SO_4$, 2.75 g.; KH_2PO_4 , 3.0 g.; Na_2HPO_4 , 7.29 g.; glucose, 5.5 g.

Medium P: $(NH_4)_2SO_4$, 2.0 g.; K_2HPO_4 , 14.0 g.; KH_2PO_4 , 6.0 g.; sodium citrate dihydrate, 1.0 g.; glucose 5.0 g.

These solutions were adjusted to pH 7.0, supplemented with $FeSO_4 \cdot 7H_2O$ (500 $\mu g.$), $CaCl_2 \cdot 2H_2O$ (10 mg.) and Mg^{2+} as indicated in the tables and figures, and sterilized by filtration through sintered glass filters. In some experiments the citrate was omitted from medium P.

The glucose used in the preparation of the above media was dissolved in water (1 g./10 ml.) and the solution passed through a column (10 cm. $\times$ 2 cm. diam.) of Chelex 100 analytical grade chelating resin (100–200 mesh; sodium hydrogen form. Bio-Rad Laboratories, Richmond, California, U.S.A.). This procedure was used also to remove bivalent cations from the mixture of amino acids that was added as a supplement to medium P in one series of experiments with *Bacillus megaterium*.

Other chemicals, all of AnalaR grade, were used without additional purification, since the degree of contamination by magnesium was small (less than $0.1 \mu\text{g./ml.}$ of the final medium).

Aerobic cultures were grown in 5 ml. volumes of medium in 25 ml. Pyrex Erlenmeyer flasks. The flasks were capped with specimen tubes or small beakers and were incubated on a mechanical shaker at 37° . For anaerobic growth, the cultures (10 ml.) were prepared in boiling-tubes plugged with cotton wool enclosed in gauze. Pasteur pipettes were inserted through the plugs into the medium and were connected through a manifold to a source of water-saturated (37°) 95 % (v/v) $N_2 + 5\% CO_2$. These cultures were incubated in a water bath at 37° . In each group of experiments, an additional uninoculated series of tubes or flasks was set up, incubated in parallel with the experimental series and then analysed to detect changes in concentration through evaporation.

Inocula (0.1 ml.) for experiments with the Gram-negative organisms were taken from fully grown cultures in media that contained initially about $0.5 \mu\text{g. } Mg^{2+}/\text{ml.}$ In these cultures the Mg^{2+} was utilized completely; the culture fluid contained no residual Mg^{2+} . As shown in the Results section, however, the Gram-positive bacilli not only required higher concentrations of Mg^{2+} to support growth, but did not assimilate the Mg^{2+} quantitatively. Thus, with these organisms, additional Mg^{2+} was transferred to each of the experimental cultures in the inoculum. In some experiments to correct for this, the excess inoculum was centrifuged and the same volume of the supernatant fluid (0.1 ml.) then added to each flask of the uninoculated control series. In others, the primary culture was centrifuged and the organisms resuspended in the same volume of Mg^{2+} -free medium to provide an inoculum of decreased Mg^{2+} content.

After incubation for 16 hr the amount of growth in the experimental series was measured turbidimetrically in a Hilger Spekker Absorptiometer on suitably diluted portions of the cultures, the extinction readings being converted to equivalent dry weight of organism ($\mu\text{g./ml.}$) by means of standard curves that were prepared for each of the organisms. The remainder of each culture was centrifuged and the supernatant fluid analysed for Mg^{2+} .

RESULTS

The data of Tables 1, 2, and 3 (left-hand series) and of Figs. 1 and 2 (aerobic cultures) show clearly that the Gram-positive bacilli *Bacillus subtilis*, (strains F3, Marburg, viscosus), *B. mesentericus*, *B. megaterium* and the Gram-negative bacteria *Aerobacter aerogenes*, *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* differed greatly in ability to concentrate Mg^{2+} . In aerobic cultures of the Gram-negative bacteria, the uptake of Mg^{2+} was quantitative at concentrations in the medium of from 0.03 to $1.5\text{--}2 \mu\text{g./ml.}$ Growth, which occurs even at very low concentrations of Mg^{2+} , increased rapidly with concentration over this range. For *E. coli* growth and Mg^{2+} -uptake followed similar curves to those shown in Fig. 1 for *S. typhimurium* and in Fig. 2 for the aerobic culture of *A. aerogenes*. With these bacteria under the given conditions of cultivation, the Mg^{2+} requirements seemed to be satisfied when the concentration of Mg^{2+} in the medium was about $4 \mu\text{g./ml.}$ Above this concentration, little increase in growth or uptake occurred. In cultures

of *P. aeruginosa* uptake of Mg^{2+} continued to increase progressively with increase in external concentration, although maximum growth was reached at about Mg^{2+} 5 $\mu g./ml.$

Table 1. *Uptake of Mg^{2+} in cultures of certain Gram-positive and Gram-negative bacteria*

The cultures were incubated for 15 hr in medium P of increasing Mg^{2+} content, as described in the text.

Mg^{2+} ($\mu g./ml.$)	Growth ($\mu g.$ dry wt. organism/ ml. culture)	Mg^{2+} uptake (%)	Growth ($\mu g.$ dry wt. organism/ ml. culture)	Mg^{2+} uptake (%)	Growth ($\mu g.$ dry wt. organism/ ml. culture)	Mg^{2+} uptake (%)
	<i>Aerobacter aerogenes</i>		<i>Escherichia coli</i> B		<i>Bacillus subtilis</i> F 3	
0.09	104	100	64	100	14	0
0.48	338	100	318	100	18	0
0.82	658	100	606	100	24	0
1.15	1120	100	710	100	60	0
1.52	1392	100	838	100	171	10.2
1.89	1600	97.9	938	87.5	332	20.2
2.93	1630	93.9	1050	82.2	366	17.5
3.87	1718	85.0	1096	70.4	346	11.9
5.74	1854	60.3	1142	50.8	412	11.3
7.61	1848	49.0	1132	42.1	428	9.7
9.49	1742	38.3	1086	34.7	470	7.9
	<i>Bacillus subtilis</i> Marburg		<i>Bacillus mesentericus</i>		<i>Salmonella</i> <i>typhimurium</i> ara 9	
0.03	15	0	30	0	92	100
0.35	15	0	27	0	318	100
0.73	14	0	40	0	552	100
1.03	51	18.2	54	0	648	100
1.47	417	48.8	70	0	758	100
1.85	520	48.9	190	3.8	1008	100
2.79	648	48.9	880	38.8	1018	68.6
3.76	672	38.4	890	35.4	1018	61.2
5.67	678	27.0	920	27.8	1048	42.9
7.56	690	21.2	1000	25.2	1268	34.5
9.52	694	15.4	—	—	908	24.5

At low Mg^{2+} concentrations complete uptake of the cation in cultures of *Aerobacter aerogenes* occurred both anaerobically and aerobically (Fig. 2). Although the maximum growth supported by the medium under anaerobic conditions was less than under aerobic conditions, the ratio of Mg^{2+} concentration to dry weight of organism at the points where Mg^{2+} uptake ceased to be complete were essentially the same.

In contrast to the Gram-negative organisms, the Gram-positive bacilli seemed unable to utilize Mg^{2+} when the concentration was less than 4×10^{-5} M, and for *Bacillus megaterium* an even higher concentration was necessary for growth. Below this minimum value, not only did the organisms not grow, but they liberated Mg^{2+} (Tables 2 and 3). Once the critical concentration of Mg^{2+} was exceeded, growth of the Gram-positive bacilli increased, at first rapidly and then more slowly, with further increases in Mg^{2+} concentration, the requirements for maximum growth (Table 2) being much greater than for the Gram-negative bacteria. Even over the initial part of this range the uptake of Mg^{2+} was not quantitative (Table 1; Fig. 1).

In cultures of *B. megaterium*, *B. mesentericus* and *B. subtilis* strain r3, all of which grew in solution P in the absence of sodium citrate, the omission of this component from the medium did not influence either growth or the utilization of Mg^{2+} . In the experiments summarized in Table 1, therefore, there was no restriction of Mg^{2+} uptake through chelation of the cation by citrate.

Fig. 1. Growth (dashed lines) and magnesium utilization (solid lines) in relation to the Mg^{2+} concentration for cultures of *Salmonella typhimurium* (●) in medium M; of *Pseudomonas aeruginosa* (○) and *Bacillus subtilis* strain viscosus (▲) in medium P.

The data of Table 3 show that after the addition of L-lysine + L-glutamic acid + L-alanine + L-arginine + DL-tryptophan to the simple basal medium, not only was the Mg^{2+} -uptake by *Bacillus megaterium* increased, but also both growth and uptake began at a lower external concentration of Mg^{2+} . These results support previous findings that the Mg^{2+} requirements for maximum growth of several Gram-positive bacilli in simple chemically defined media are decreased by the addition of various amino acid mixtures (Webb, 1951). The presence of several amino acids appeared to be necessary before this decrease took place; no single amino acid was found to be essential.

The liberation of cellular Mg^{2+} that was observed on transfer of inocula of Gram-positive bacilli to a Mg^{2+} -deficient medium, also occurred with Gram-negative bacteria, but with these latter organisms the liberated cation was re-utilized rapidly. Thus when *Escherichia coli* and *Bacillus megaterium* were grown in the

presence of Mg^{2+} 10 $\mu g./ml.$ and then resuspended at 37° in media deficient with respect to Mg^{2+} , both organisms liberated about 20% of their Mg^{2+} (Table 4). On further incubation of the suspensions of *E. coli* the liberated Mg^{2+} was re-assimilated rapidly, and when the initial suspension concentration was low, some growth occurred

Table 2. *Growth and Mg^{2+} uptake in cultures of Bacillus subtilis and B. mesentericus in relation to the magnesium content of the medium*

The inocula for these cultures were grown in the chemically defined medium P containing Mg^{2+} 5 $\mu g./ml.$ The bacteria were separated by centrifugation after 18 hr and resuspended in the same volumes of Mg^{2+} -free medium. Each experimental flask was inoculated with 0.1 ml. of the appropriate suspension, and incubated for 16 hr at 37° .

<i>Bacillus subtilis</i> F8			<i>Bacillus mesentericus</i>		
Mg^{2+} ($\mu g./ml.$)	Growth ($\mu g.$ dry weight organism/ml. culture)	Mg^{2+} uptake (%)	Mg^{2+} ($\mu g./ml.$)	Growth ($\mu g.$ dry weight organism/ml. culture)	Mg^{2+} uptake (%)
0.03	6	0*	0.08	5	0*
9.3	720	15.3	1.88	34	3.9
18.9	732	9.8	3.86	1075	37.3
28.9	754	8.3	5.87	1280	45.8
38.1	756	7.5	7.58	1510	26.7
47.8	740	6.4	9.68	1500	30.2
57.3	696	5.7	16.1	1520	12.1
67.0	672	5.1	21.0	1420	9.3
76.7	674	5.4	31.4	1400	10.0
86.4	644	5.7	41.0	940	5.1

* In both of these cultures the magnesium content was greater than in the corresponding, uninoculated control. The liberated Mg^{2+} amounted to 0.048 $\mu g./ml.$ and 0.082 $\mu g./ml.$ in the *B. subtilis* and *B. mesentericus* cultures, respectively.

Table 3. *Influence of an amino acid supplement on the growth and magnesium-utilization of Bacillus megaterium KM in a mineral salts glucose medium*

Measurements were made on both cultures after incubation for 16 hr at 37° .

Basal medium P				Basal medium + supplementary amino acids*			
Mg^{2+} ($\mu g./ml.$)	Growth ($\mu g.$ dry wt. organism/ ml. culture)	Mg^{2+} uptake or liberation		Mg^{2+} ($\mu g./ml.$)	Growth ($\mu g.$ dry wt. organism/ ml. culture)	Mg^{2+} uptake or liberation	
		($\mu g./ml.$)	(%)			($\mu g./ml.$)	(%)
0.03	24	+0.14†	0	0.05	9.6	+0.08	0
0.35	24	+0.21	0	0.38	9.6	+0.16	0
0.73	26	+0.26	0	0.75	9.6	+0.10	0
1.03	31	+0.14	0	1.13	16	+0.10	0
1.47	31	+0.08	0	1.50	18	0.03	2.0
1.85	35	+0.03	0	1.87	282	0.50	26.8
2.79	50	+0.04	0	2.82	1880	1.07	38.0
3.76	76	+0.05	0	3.75	2032	2.07	55.2
5.67	1010	1.15	20.3	—	—	—	—
7.56	1040	1.71	22.6	—	—	—	—
9.52	1060	2.04	21.4	—	—	—	—

* L-Lysine + L-glutamic + L-alanine + L-arginine (all 4×10^{-3} M) + DL-tryptophan (3×10^{-3} M).

† The + sign signifies liberation of magnesium.

(Table 4). With *B. megaterium* under these conditions there was partial re-utilization of the Mg^{2+} in the first 20 min. of incubation, but thereafter, presumably as a result of autolysis, even greater amounts of the Mg^{2+} were liberated and the bacterial mass decreased. The initial re-utilization and subsequent liberation of Mg^{2+} were, respectively, greater and less at high (equiv. 2 mg. dry wt. organism/ml.) than at low (equiv. 0.5 mg. dry wt./ml.) suspension concentrations (Table 4).

Table 4. *Magnesium liberation from Escherichia coli (Gram-negative) and from Bacillus megaterium (Gram-positive)*

Cultures of *E. coli* and *B. megaterium* in medium M (400 ml.; Mg^{2+} 10 μ g./ml.) and medium P (400 ml.; Mg^{2+} 10 μ g./ml.), respectively, were grown with shaking at 37°, and harvested after 11 hr (Mg^{2+} uptake in *E. coli* culture = 2.24 μ g./ml.; in *B. megaterium* culture = 1.08 μ g./ml.) The organisms were washed twice with 0.9% NaCl (total vol. 150 ml.; Mg^{2+} contents of washings from *E. coli* and *B. megaterium* were 0.99 and 0.47 μ g./ml., respectively) and resuspended in 0.9% NaCl at about equiv. 50 mg. dry wt. organism/ml. Both suspensions, which contained 1.40 (*E. coli*) and 1.03 (*B. megaterium*) Mg^{2+} μ g./mg. dry wt. organisms, were diluted with the appropriate Mg^{2+} -free medium, prewarmed to 37°, and incubated with shaking at this temperature. Immediately after preparation, and at intervals thereafter, samples of the suspensions were removed, cooled in crushed ice and centrifuged, the supernatant fractions being analysed for Mg^{2+} . Dry weights were determined turbidimetrically on other portions of the suspensions after dilution.

Period of incubation (hr) at 37°	Initial suspension concentration (mg. dry wt. organism/ml.)					
	0.5			2.0		
	Suspension concen- tration*	Mg ²⁺ leakage		Suspension concen- tration*	Mg ²⁺ leakage	
		μg./ml.	% of total Mg ²⁺		μg./ml.	% of total Mg ²⁺
<i>Escherichia coli</i>						
0	476	0.136	20.4	1960	0.56	19.7
0.33	—	0.052	7.8	—	0.06	2.2
2.2	772	0.0	0.0	1820	0.0	0.0
4.2	900	0.0	0.0	1880	0.0	0.0
6.0†	—	0.0	0.0	—	0.0	0.0
<i>Bacillus megaterium</i>						
0	436	0.14	31.2	1880	0.36	18.6
0.33	—	0.10	22.2	—	0.26	13.5
2.2	428	0.20	44.6	1640	0.20	10.4
4.2	404	0.28	62.4	1620	0.28	14.5
6.0†	—	0.39	86.9	—	0.56	29.0

* Equiv. μ g. dry wt. organism/ml.

† At this time only intact normal rods were present in the *E. coli* suspensions, whereas the suspensions of *B. megaterium* contained much debris and many very swollen organisms.

As shown by Fig. 3, the liberation of Mg^{2+} from *Aerobacter aerogenes* in a Mg^{2+} -free medium seemed to occur by diffusion, whereas the re-utilization of Mg^{2+} was an energy-dependent process. Thus, about the same amounts of Mg^{2+} was liberated from suspensions of equal dry weight at 0°, 10°, 20° and 30°. The rate of re-utilization (Mg^{2+} μ g./hr/mg. dry wt. organism) which, under the defined experimental conditions, was essentially zero at 0°, increased with temperature, and was 1.10 at 10°, 2.16 at 20°, and, with reservations because of the extrapolation, 5.32 at 30°.

Fig. 2

Fig. 3

Fig. 2. Growth (dashed lines) and magnesium utilization (solid lines) in relation to the Mg^{2+} concentration in aerobic (●) and anaerobic (△) cultures of *Aerobacter aerogenes* in medium M.

Fig. 3. Mg^{2+} liberation from, and re-utilization by *Aerobacter aerogenes* when suspended with a metabolizable substrate in the absence of exogenous Mg^{2+} at pH 6.0, at 0° (▼), 10° (■), 20° (▲), 30° (●). For these measurements, mixtures of 0.1 M-potassium phosphate buffer (pH 6.0; 7.5 ml.), 0.04 M-Na pyruvate (2.5 ml.) and water (5.0 ml.) were brought to the above temperatures, and a suspension of *A. aerogenes* (5.0 ml.; equiv. 10 mg. dry wt. organism/ml. in 0.9 % w/v, NaCl) added to each. The suspensions were incubated with shaking for 20 min., portions of each being withdrawn at 5 min. intervals, cooled rapidly to 0° and centrifuged. Magnesium was determined in the supernatant solutions as described in Methods section.

DISCUSSION

The preceding results show that the Gram-positive bacilli were unable to utilize Mg^{2+} when the concentration of this ion was low in a simple medium, otherwise adequate for growth. In media of increasing Mg^{2+} content, the onset of growth was correlated with Mg^{2+} uptake, but the concentration at which assimilation began seemed to vary with the species (Table 1). Even when the Mg^{2+} concentration was sufficient to support half-maximal growth, less than 50 % of the available Mg^{2+} was assimilated. In contrast, the uptake of Mg^{2+} in cultures of all the Gram-negative bacteria examined was quantitative over the range where growth was limited by the Mg^{2+} concentration, and even when this concentration was extremely low, some increase in dry weight of organisms occurred (Table 1). These observations on cultures of Gram-negative bacteria support and extend to other organisms the findings of Tempest, Hunter & Sykes (1965) about the Mg^{2+} -limited growth of *Aerobacter aerogenes*.

Both Gram-negative bacteria and Gram-positive bacteria liberated Mg^{2+} , apparently by diffusion, when suspended in media free from this cation (Table 4). The Gram-negative bacteria, however, rapidly re-utilized the liberated Mg^{2+} , whereas with the Gram-positive bacilli, assimilation was incomplete, and ultimately the cellular structure disintegrated. Re-utilization required a metabolizable substrate, which may be either endogenous (unpublished work) or exogenous (Fig. 3),

but no other components of the growth medium. The action of various metabolic inhibitors on this energy-dependent utilization of Mg^{2+} , both alone and in relation to the uptake of toxic bivalent heavy metals, will be described subsequently.

The findings discussed above account for the difference between the Mg^{2+} requirements of Gram-positive bacteria and those of Gram-negative bacteria for growth in simple chemically defined media (Webb, 1949*a*). They also suggest that the effects of Mg^{2+} deficiency on the structure and function of the ribosomal aggregates may be more pronounced in the Gram-positive organisms than in the Gram-negative ones. This would explain previous observations (Webb, 1953) on the decrease in protein content of Gram-positive but not Gram-negative bacteria during growth in Mg^{2+} -deficient media.

Thus far, studies on the effects of Mg^{2+} deficiency on the ribosomal structures of bacteria appear to have been confined to Gram-negative organisms. Kennell & Magasanik (1962), for example, observed that Mg^{2+} starvation greatly decreased the ribosome content of an arginine- and guanine-requiring mutant of *Aerobacter aerogenes*. Also, McCarthy (1962) found that log-phase *Escherichia coli* cells remained viable when incubated for 20 hr in a medium without Mg^{2+} , but show an almost complete loss of ribosomes, and even after 6 hr there was a considerable decrease in the content of 70 S particles. In this connexion it is interesting that the results of Table 4 show that, after the re-utilization of the Mg^{2+} initially liberated from *A. aerogenes* on transfer to the Mg^{2+} deficient medium, there was no further loss of the cation over a 6 hr period. As the integrity of ribosomes depends not only upon the concentration of Mg^{2+} , but also on the relative concentrations of other ions, particularly K^+ and Na^+ (e.g. Elson, 1961) it seems possible, therefore, that the disappearance of these structures from Gram-negative cells in response to Mg^{2+} deficiency may be due to the unfavourable intracellular ratio of monovalent cations to Mg^{2+} , rather than to the loss of Mg^{2+} from the organisms.

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