The Influence of Magnesium on Cell Division

2. The Effect of Magnesium on the Growth and Cell Division of Various Bacterial Species in Complex Media

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SUMMARY: Magnesium is essential for the normal cell division of bacilli in complex media. Under conditions of magnesium deficiency or magnesium excess, cell division is inhibited and filamentous cells may be formed. Under the same conditions there is no appreciable interference with the division of chromatinic bodies. The magnesium requirements of the Gram-positive bacteria are considerably greater than those of the Gram-negative bacteria, possibly because the former incorporate magnesium into the structure of the Gram complex.

In a previous communication (Webb, 1948*a*) it was shown that magnesium was essential for the normal cell division of *Clostridium welchii*. In complex media deficient in ionic magnesium, *Cl. welchii* grew in the form of long filaments, which reverted to cells of normal morphology when subcultured in a medium containing 0.0015 % (w/v) magnesium ion. In extending these studies, the influence of magnesium on the growth and division of various other species of bacteria in complex media has been determined. The results, described here, suggest that magnesium is essential for the normal cell division of all the rod-shaped bacteria studied, but that fundamental differences exist between the magnesium requirements of Gram-positive and Gram-negative organisms.

MATERIAL AND METHODS

The majority of the bacteria studied were obtained from the National Collection of Type Cultures and are indicated in the text by the N.C.T.C. 1936 catalogue number following the name of the organism. The remaining cultures were from a collection maintained in this department.

Magnesium analyses were made gravimetrically as previously recorded (Webb, 1948*a*). Although these analyses were tedious and required about 2 g. dry cells for each determination in duplicate, the method was more accurate and more reproducible than colorimetric determinations.

A peptone water medium containing 2 % (w/v) peptone (Evans Medical Supplies, London) was made deficient in ionic magnesium by precipitation of this element as magnesium ammonium phosphate as previously described (Webb, 1948*a*). By this means 94 % of the total magnesium present in the peptone was removed and the final medium contained *c.* 0.00003 % (w/v) Mg.

When Mg-deficient liquid media were solidified with agar, the magnesium present in the agar was utilized by bacteria. Ignition of agar (Ward, Blenkinsop and Co.) gave 1.34 % ash, which contained 2.65 % magnesium. When magnesium, together with other metallic ions (K^+ , Na^+ , Ca^{++} , Fe^{+++} , Sn^{++}), was removed by electrodialysis at 45°, the agar completely lost its setting properties.

Tin, iron, calcium and magnesium were completely removed when 0.1 N hydrochloric acid (2 l.) was slowly passed through a column (2 cm. diam.) of agar (25 g.). After 4 days this modified agar was transferred to a Buchner funnel and washed first with distilled water until free from mineral acid, and then with 0.2 M-phosphate buffer pH 7.8. In contrast to electro dialysed agar, 4 % (w/v) solutions of this washed agar in the magnesium-deficient broth together with 0.005 % (w/v) Ca^{++} set to a rigid gel.

EXPERIMENTAL

Influence of magnesium on growth and cell division

Clostridium and *Bacillus* spp.; *Gram-positive*. The abnormal, filamentous morphology of Gram-positive species of *Clostridium* and *Bacillus* grown in the magnesium-deficient medium at 37° (Table 1) revealed that magnesium was essential for normal cell division of these organisms. The formation of filaments

Table 1. *Growth of Bacilliaceae in a magnesium-deficient peptone medium*

Cultures in 'ammonia precipitated' Evans peptone broth grown for 18 hr. at 37°; magnesium concentration ≥ 0.00003 %.

Organism	Morphology
<i>Cl. tertium</i>	Filaments and chains
<i>Cl. sporogenes</i>	Filaments and chains
<i>B. subtilis</i> var. <i>viscosus</i> (No. 2587)	Long filaments
<i>B. subtilis</i> (No. 3610)	Filaments, distorted filaments, chains and some normal cells
<i>B. polymyxa</i> (No. 1380)	Filaments and shorter rods
<i>B. vulgatus</i> (No. 2588)	Long filaments together with normal cells in chains
<i>B. mycoides</i> (No. 2602)	Long filaments together with normal cells in chains
<i>B. megatherium</i> (No. 2605)	Filaments and longer cells in chains
<i>B. anthracis</i>	Long filaments and chains of normal rods extending over several fields

was not altered by serial subculture of the bacilli in the magnesium-deficient medium, but these filamentous cells gave rise to cells of normal appearance when subcultured in a medium containing Evans peptone (2 % w/v), NaCl (0.5 %, w/v) and glucose (0.2 %, w/v). The more abundant growth that occurred in this medium presumably indicates that magnesium plays some part in protoplasmic synthesis as well as in cell division.

In agreement with the results obtained with *Cl. welchii* (Webb, 1948*a*), no change in morphology occurred when filaments from 14 hr. cultures of *B. subtilis*, *B. mycoides* and *B. vulgatus* in the magnesium-deficient medium were incubated at 37° either in 0.1 % (w/v) magnesium sulphate, or in autolysed normal cultures of the organisms. On the magnesium-deficient solid medium the aerobic bacilli either failed to grow or grew with difficulty. *B. megatherium*, for example, formed scattered irregular terraced colonies with ragged or lobate margins, which were composed of long cells in chains.

In agreement with the studies of Hinshelwood (1946) on the growth of *Bact. lactis aerogenes* (*Aerobact. aerogenes*) the growth temperature had a pronounced influence on the production of filaments in the liquid medium. Thus, in cultures

of *Cl. tertium*, *B. subtilis* var. *viscosus* and *B. mycoides* incubated at 18°, the growth was poor and was composed of many normal cells together with some chains and filaments. Better growth occurred in cultures of *Cl. welchii*, *Cl. tertium* and *B. anthracis* at 25° and, in each case, the cells were in long chains. At these lower temperatures, where growth was slow, the final stationary population appeared to be sharply limited by the magnesium concentration and, in consequence, the incidence of cells of abnormal length was decreased.

The influence of magnesium concentration on the growth of the bacilli was strikingly illustrated when *Cl. welchii*, *Cl. tertium*, *B. subtilis* var. *viscosus*, *B. vulgaris* and *B. mycoides* were cultivated in complex media at 37°. As the magnesium concentration was increased from that of the 'ammonia precipitated' Evans peptone medium, the amount of growth increased visibly, while the morphological appearance of the cells changed from filaments to chains and then to isolated normal rods. On increasing the concentration further, the amount of growth progressively decreased and the normal rods were replaced by chains and finally by filaments (Fig. 1). The inhibitory effect of higher magnesium concentrations on cell division in particular is in accordance with the fact that enzymes activated by metallic ions in low concentrations are also inhibited by the same ions when the concentration exceeds a certain limit (cf. Clark, 1938).

Lactobacillus helveticus, *L. arabinosus* and *Kurthia zenkeri*; *Gram-positive*. Filamentous forms of *Kurthia zenkeri* (*Zopfius zenkeri*, No. 404), *Lactobacillus helveticus* and *L. arabinosus* were obtained in magnesium-deficient media. Magnesium is therefore essential for the normal cell division of these bacteria. The magnesium requirements of the lactobacilli were somewhat less than those of the Gram-positive bacilli and clostridia, since the poor growth in the magnesium-deficient medium at 37° was, especially in the case of *L. helveticus*, composed mainly of long chains, and a predominance of filaments was only obtained when the cultures were incubated at 40°. Excess magnesium (0.05–0.1 % w/v) also inhibited cell division and induced the production of filamentous forms.

Gram-positive and Gram-negative cocci. No abnormal morphology was observed in cultures of *Staphylococcus citreus* (B 9), *Staph. albus*, *Gaffkya tetragena* (*Micrococcus tetragenus*, No. 951), *Streptococcus pyogenes* (No. 2400), *Strep. faecalis* and three *Neisseria* species in the magnesium-deficient liquid medium. Several of these cocci were maintained by subculture for over a year in this medium without any variation in size. Furthermore, no changes in morphology occurred when the organisms were cultivated in the presence of excess magnesium. On the other hand, magnesium was essential for the growth of the Gram-positive and Gram-negative cocci, since all the strains studied grew with difficulty under the conditions of magnesium deficiency or excess, and on the solid magnesium-deficient medium only *Staph. citreus* was capable of growth.

Gram-negative rods. In contrast to the Gram-positive rods, better growth and normal cell-division occurred in magnesium-deficient cultures at 37° of the following strains: *Pseudomonas aeruginosa* (No. 1999), *Ps. prunicola* (No. 3370),

Chromobacterium violaceum (No. 2537), *Serratia marcescens* (*Chromobact. prodigiosum*) (No. 2302), *Proteus vulgaris* (No. 401), *Escherichia coli* commune (No. 86), *E. coli* var. *acidilactici* (*Bact. acidi lactici*, No. 123), *Aerobacter aerogenes* (*Bact. lactis aerogenes*), *Aerobacter cloacae* (*Bact. cloacae*, No. 408) and *Alcaligenes faecalis* (*Bact. faecalis alcaligenes*, No. 415). Only occasional filamentous cells were observed during a minimum of ten subcultures. The only marked change

Fig. 1

Fig. 2

Fig. 1. Diagrammatic representation of the effect of increasing magnesium concentration on the morphology of *Clostridium welchii*, *Cl. tertium*, *Bacillus subtilis* var. *viscosus*, *B. vulgatus* and *B. mycoides*.

Fig. 2. Diagrammatic representation of the filamentous forms of *Clostridium welchii*, *Bacillus vulgatus* and *Serratia marcescens* stained to show the transverse cell walls.

was the increased polysaccharide synthesis in cultures of *Ps. prunicola* and *Aerobacter aerogenes*. The same change was observed in cultures of *Cl. welchii* in the magnesium-deficient medium (Webb, 1948*a*), and Shear & Turner (1943) found that the addition of magnesium salts to cultures of *Serratia marcescens* resulted in decreased yields of their 'haemorrhage-producing' polysaccharide.

It would appear, as with the Gram-positive organisms, that magnesium stimulated the growth of these Gram-negative organisms; the growth, as determined by opacity measurements, was invariably less in the magnesium-deficient medium than in the normal 2% peptone medium. Furthermore, Young, Begg & Pentz (1944) have shown that magnesium is essential for the normal growth of *E. coli*.

On subculture in media of greater magnesium concentration these organisms grew as long filaments. The concentration of magnesium (0.05–0.1% w/v) required to induce this change was considerably less than was the case with the Gram-positive bacteria. Indeed, higher magnesium concentrations (0.25, 0.5% w/v) often inhibited the growth of the Gram-negative species. In agreement with this, Kishimo (1927) observed that *Bact. typhosum* grew as chains and

filaments in a chemically defined medium containing 1 % magnesium sulphate while in 2 % peptone water containing magnesium the organism formed long chains. These morphological variations were not produced by other salts such as K_2HPO_4 , ammonium lactate, $CaCl_2$ and NaCl.

Division of filaments of *Ps. prunicola* and *Serratia marcescens* from cultures in the complex medium containing 0.05 % (w/v) Mg^{++} could not be induced by incubating the washed cells suspended in sterile saline at 37°.

From these results it was concluded that magnesium was equally essential for the normal cell division of the Gram-negative rods, but the critical concentrations were lower than those required by the Gram-positive bacteria. Studies of *Cl. welchii* and other Gram-positive organisms (Henry & Stacey, 1946) showed that the surface complex responsible for the Gram stain contains magnesium ribonucleate, and that this complex is absent from the Gram-negative bacteria. The Gram complex removed from *Cl. welchii* by extraction with sodium cholate contained 3.2 % Mg^{++} , whereas the residual Gram-negative cytoskeletons contained 0.1 % Mg^{++} (Henry, Stacey & Teece, 1945). From these results it is calculated that about 70 % of the total magnesium present in *Cl. welchii* (0.35 %, Webb, 1948*a*) is actually in the Gram complex. Thus if magnesium fulfils the dual role of a structural element and an enzyme activator in Gram-positive organisms, whereas in Gram-negative organisms it functions as an enzyme activator only, it is obvious that the magnesium requirements of Gram-negative bacteria will be considerably less than the requirements of Gram-positives.

The analysis (Table 2) of cells harvested from a peptone water medium of constant composition shows that, of the bacteria examined, the Gram-positive bacteria contained a higher percentage of magnesium than did the Gram-negative cells. Furthermore, when the bacterial cells were killed by heat under the conditions previously described (Webb, 1948*b*), dialysed against running tap water for 48 hr. and then slowly passed through a column of mixed ion-exchange resins (De-acidite C and ZeoKarb H.I.P. in the ratio 6:1) the Gram-positive cells retained a percentage of their magnesium, whereas with one exception, the Gram-negative cells retained none (Table 2). This result was not an artefact due to the greater magnesium concentration of Gram-positive cells, because the suspension of micrococci (T 38) was passed twice through the ion-exchange resin column before analysis. In these experiments, killed cells were used in order to avoid autolytic changes during dialysis. With certain Gram-positive bacteria, such as *Cl. welchii* and *L. plantarum*, there was a marked tendency for the cells to become Gram-negative when killed by heat unless the previously described precautions were observed. The magnesium of those cells which became Gram-negative by extraction with sodium cholate was completely removed by the ion-exchange resins (Table 2).

From these results it is concluded that magnesium is more tenaciously bound in Gram-positive than in Gram-negative bacteria. Although this bound magnesium does not correspond to the total magnesium of the Gram complex, it appears to be held by the complex, since magnesium is readily removed from the Gram-negative forms of *Cl. welchii*.

Table 2. *The removal of magnesium (and other ions) from killed Gram-positive and Gram-negative bacteria by mixed ion-exchange resins*

Organism	Ash (percentage of dry wt. of cells)		Mg ⁺⁺ (percentage of dry wt. of cells)	
	Normal cells	Cells passed through ion-exchange resins	Normal cells	Cells passed through ion-exchange resins
Gram-positive				
<i>Cl. welchii</i>	7.3*	2.0*	0.35*	0.02
<i>Micrococcus</i> (T 38)	8.25	3.55	0.72	0.037
<i>B. subtilis</i>	9.8	—	0.70	—
<i>L. plantarum</i>	6.3	3.1	0.63	0.03
<i>Strept. faecalis</i>	7.5	3.4	0.68	0.034
Gram-negative				
<i>Chromobact. violaceum</i>	5.20	2.1	0.26	Nil
<i>Aerobact. cloacae</i>	6.68	3.00	0.25	0.019
<i>Aerobact. aerogenes</i>	5.16*	3.17	0.01*	Nil
<i>Ps. aeruginosa</i>	7.26	5.1	0.17	Nil
<i>Ps. prunicola</i>	3.6	1.4	0.066	Nil
<i>Cl. welchii</i> rendered Gram-negative by extraction with 2% sodium cholate	—	1.8	0.1†	Nil

* Webb (1948a).

† Henry, Stacey & Teece (1945).

Application of specific staining methods

Filaments of *Cl. welchii*, *Cl. tertium* and *B. polymyxa* from cultures in magnesium-deficient media, and filaments of *Ps. prunicola* and *Serratia marcescens* from cultures in media containing excess magnesium, were stained by Robinow's (1944) HCl-Giemsa method. They showed chromatinic bodies regularly spaced throughout the cytoplasm. Such cultures were 'old' (12–14 hr.) in the sense employed by Robinow, since it was considered important to distinguish filaments produced under these conditions and the shorter filamentous cells which are normally observed during the early phases of active growth of the rod-shaped bacilli (cf. Clark & Ruehl, 1922). When stained by Robinow's tannic acid-crystal violet method, transverse cell walls were observed. In some cases, these were regularly spaced along the filament. In others, only a few transverse septa were observed (Fig. 2) which divided the filaments into sections of different lengths containing varying numbers of chromatinic structures (cf. Klieneberger-Nobel, 1944).

DISCUSSION

From the observations described here, it is concluded that the mechanism of cell division is the same in the Gram-positive and Gram-negative rod-shaped bacteria, and requires magnesium for its normal function. The differences between the magnesium requirements of the Gram-positive and Gram-negative organisms is attributed to the fact that the former also require magnesium for the formation of the Gram complex.

The stage in division which requires magnesium remains to be determined. It appears that magnesium is not essential for the division of the chromatinic bodies, for these structures are regularly distributed throughout the length of filamentous cells. If these chromatinic bodies represent true nuclei, then cell division, in the sense of the complete fission of the bacterial cell, is not a necessary consequence of nuclear division. Robinow (1945) has already shown that the onset of cell division in the rod-shaped bacteria is not related to any particular stage in the division of the chromatinic bodies. Magnesium does not appear to be primarily concerned with the formation of transverse cell-walls, since the process is only partially inhibited by magnesium deficiency or magnesium excess. The fact that the Gram-positive bacilli change from filaments to chains as the magnesium content of the medium increases towards the concentration optimal for normal cell division (Fig. 1), suggests that magnesium is involved at a stage in division between the splitting of a transverse cell-wall and the separation of the daughter cells.

The fragmentation of filamentous cells resulting either from magnesium deficiency or magnesium excess, was not observed in cultures and could not be induced artificially. In this respect, these filamentous cells differ from the shorter filaments which are observed in cultures of rod-shaped organisms during the early phase of active growth and which do subsequently divide.

The cell division of the Gram-positive and Gram-negative cocci differs from that of the rod-shaped organisms, since conditions of either magnesium deficiency or magnesium excess, although markedly inhibitory to growth, fail to produce any changes in the morphology of micrococci, sarcinae and neisseria. The streptococci examined here did not show any changes in morphology, although Bisset (1948) has observed filamentous cells in streptococcal cultures.

Presumably either many metabolic activities occur at the surface of the bacterial cell, or the material essential for growth must diffuse through the cell surface. Consequently, a limitation will be imposed upon the size of a bacterial cell by the surface/volume (S/V) relationship, since, in general, as the size of an organism increases, S/V and, therefore, metabolism per unit volume, decreases. For a spherical coccus, $S/V=3/r$, where r is the radius of the cell, and for a cylindrical 'square-ended' bacillus $S/V=3/d+2/l$, where d is the diameter and l the length of the organism. When bacilli grow in the form of filaments, as l increases d tends to decrease (cf. Webb, 1948*a*). Hence it follows that, in so far as such calculations are justified, any change in the dimensions of a cell will be of greater significance in the case of a coccus than a bacillus. Such reasoning may, in part at least, explain the fact that magnesium deficiency or magnesium excess predominantly influences the growth, and not the morphology, of the cocci.

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