The Influence of Magnesium on Cell Division

4. The Specificity of Magnesium

By M. WEBB

*Chemistry Department, The University, Edgbaston, Birmingham 15, and
The Strangeways Research Laboratory, Cambridge*

SUMMARY: Magnesium exerts a specific function in (a) the formation of the Gram complex, and the activation of cell division in certain Gram-positive bacteria, and (b) activation of bacterial growth. Of a series of divalent metallic ions, only manganese is capable of partially replacing magnesium in the above processes.

In previous communications (Webb, 1948, 1949*a, b*) magnesium was shown to be essential for the growth and cell division of the rod-shaped bacteria. In complex (i.e. peptone-water) media, conditions of magnesium deficiency or excess were found to inhibit the process of cell division, whereas in simple chemically-defined media, these conditions predominantly limited growth (i.e. synthesis of cell substance). The magnesium requirements of Gram-positive organisms in both simple chemically-defined media, and complex media were found to be considerably greater than the requirements of Gram-negative bacteria, and it was suggested that this difference may be due, in part at least, to the fact that the former contain magnesium as an essential component of the Gram-positive complex (cf. Henry & Stacey, 1946).

The scope of the work thus far recorded has been somewhat limited by the absence of a rapid and accurate gravimetric micro-method for the estimation of magnesium in bacterial cells and culture media. It appeared that the extension of these studies, as indicated elsewhere (Webb, 1949*c*, 1950), could be greatly facilitated by the replacement of magnesium by a functionally equivalent metallic ion for which a radio-active isotope could be used as a tracer. The magnesium isotope itself is unsuitable for this work owing to its extremely short half-life period.

The present paper describes the results of experiments in which other divalent metallic ions were examined for their ability to replace magnesium in the formation of the Gram complex, the activation of the processes of cell division in certain Gram-positive bacteria and the activation of cellular synthesis and metabolism.

EXPERIMENTAL

Most of the metallic ions studied had radii similar to that of the magnesium ion (Table 1) and, with the exception of manganese were used in the form of their sulphates or chlorides (Analar grade) without further purification. As the manganous and magnesium ions are isomorphous, manganese salts usually contain a relatively high percentage of magnesium. Pure manganous sulphate

therefore was prepared from magnesium-free potassium permanganate by the following reactions:

The replacement of magnesium by other metallic ions was studied as follows.

Formation of the Gram complex

Washed cells from 3 l. of an 18 hr. culture of *Clostridium welchii* in 2 % (w/v) Evans's peptone water containing 0.5 % (w/v) glucose, were suspended in 2 % (w/v) sodium cholate (200 ml.) and kept at 60° until Gram-negative (16–18 hr.). These Gram-negative 'cytoskeletons' (Henry & Stacey, 1946) were collected by centrifuging, washed twice with water and suspended in 0.85 % (w/v) sodium chloride (100 ml., containing 2 % (v/v) of 40 % formaldehyde). After 16 hr. at room temperature the suspension was centrifuged, the deposit washed three times with water, and then suspended in a 2 % (w/v) solution of purified sodium ribonucleate (100 ml.; Henry & Stacey, 1946). Two ml. of this suspension were added to each of a series of test-tubes containing solutions (2 ml.) of the various metallic ions (Table 1), together with sodium ethyl mercurithio-salicylate (1:1000, 0.1 ml.). After 24 hr. at room temperature the approximate percentage of Gram-positive cells in each suspension (Table 1) was determined by the examination of stained smears.

Table 1. *The ability of other metallic ions to replace magnesium in the formation of the Gram complex of Clostridium welchii*

(Reduced Gram-negative cytoskeletons of *Cl. welchii* were suspended in 1% (w/v) sodium ribonucleate in the presence of the given metallic ion. Representative results below show the approximate percentage of Gram-positive cells observed in a series of stained smears after 18–24 hr. at room temperature.)

Metallic ion (0.6×10^{-3} g. ions/l.)	Ionic radius (A.)* according to		Gram-positive cells (% approx.)
	Goldschmidt	Pauling	
Mg ⁺⁺	0.78	0.65	80–100
Ca ⁺⁺	0.99	—	10
Mn ⁺⁺	0.91	0.80	80–100
Zn ⁺⁺	0.83	0.74	0
Ni ⁺⁺	0.78	0.69	0–10
Co ⁺⁺	0.82	0.72	0
Fe ⁺⁺	0.83	0.75	0
Be ⁺⁺	0.34	0.31	—†
None (control)	—	—	0

* Taken from *Handbook of Chemistry and Physics* (30th ed. 1947, p. 2628), Chemical Rubber Publishing Co.

† There was complete precipitation of ribonucleic acid by beryllium. It will be shown elsewhere that beryllium resembles lanthanum in its ability to form highly insoluble salts with both ribo- and deoxyribonucleic acids. This property may have some significance with regard to the toxicity of beryllium.

Activation of cell division

A peptone medium deficient in magnesium was prepared as described previously (Webb, 1948). In this medium (L; Mg⁺⁺ content *c.* 0.0001 %, w/v),

Gram-positive bacilli and clostridia formed long filamentous cells. These filaments gave cells of normal morphology when subcultured in the same medium supplemented with 0.0015 % (w/v) magnesium (i.e. 6.17×10^{-4} g. ions/l.; medium M).

The ability of other divalent metallic ions to activate cell division was determined when inocula of filamentous cells from magnesium-deficient cultures (*Cl. welchii*, *Cl. tertium*, *Bacillus mycoides*, *B. vulgatus* and *B. subtilis* var. *viscosus*) were subcultured in medium M in which the Mg^{++} was replaced by other metallic ions at the same ionic concentration. When growth occurred, the cultures were centrifuged after 24–48 hr. at 37° and the morphological characteristics of the daughter cells determined in stained smears.

Under these conditions, the ions Co^{++} , Ni^{++} , Zn^{++} and Be^{++} did not replace Mg^{++} in the cell-division process. Moreover, these ions, even in the low concentrations employed, partially inhibited the growth of the organisms studied, the toxicity of the ions increasing in the above order. The filamentous cells observed in cultures of both the clostridia and bacilli containing Zn^{++} and Co^{++} were considerably longer than those observed in control cultures of the same organisms in medium L. The addition of these ions to medium M, however, failed to produce filaments. Thus, although Zn^{++} and Co^{++} are unable to replace Mg^{++} in cell division, it appears possible that, because of their similarity to the magnesium ion in valency and ionic radius, they may exhibit a type of competitive antagonism and prevent the uptake by the growing organisms of the residual Mg^{++} in the Mg^{++} deficient medium.

The growth of *B. subtilis* var. *viscosus*, *B. mycoides* and *B. vulgatus* was stimulated by Mn^{++} almost to the same extent as by Mg^{++} at the same concentration. The cells from these cultures varied in morphology from almost coccoidal forms to normal rods. Filamentous cells, however, were not observed.

With *Cl. welchii* and *Cl. tertium* the addition of Mn^{++} to the medium increased the amount of growth more than the addition of Mg^{++} . However, whereas the cells from the cultures containing the latter were of normal morphology, those from cultures containing Mn^{++} were filaments, and appeared essentially similar to those observed in the control cultures (medium L).

From these results it appeared that in the anaerobic cultures the divalent manganous ion was oxidized to the tri- or tetravalent state. This oxidation might conceivably enhance the growth of the anaerobes by maintaining reducing conditions, but, at the same time, would convert the manganese into a form in which it no longer resembled the magnesium ion. Accordingly, medium L (20 ml.) was supplemented by the addition of reducing agents (thiolacetic acid, 0.1 ml.; cysteine hydrochloride, 2 mg.; or L-ascorbic acid, 2 mg.). Although under these conditions, the stimulation of growth of either *Cl. welchii* or *Cl. tertium* which followed the addition of Mn^{++} to the medium was less than that which occurred when Mg^{++} was added, the final population was composed of short rods of normal morphology and filamentous cells were not observed.

Activation of growth in a simple chemically-defined medium

Of a number of chemically-defined media studied, that described by Koser & Rettger (1919), when containing 40 p.p.m. magnesium, supported the growth of all the organisms examined (*B. vulgatus*, *B. mycoides*; *Aerobacter cloacae*, *Escherichia coli commune* and *Pseudomonas aeruginosa*) and appeared most suitable for comparative purposes. The medium was prepared free from Mg^{++} , as previously described (Webb, 1949*b*). The ability of other metallic ions to initiate growth in this medium was determined over the concentration ranges of 0 to 4.12×10^{-4} g. ion/l. (Fig. 2) and 0 to 4.12×10^{-3} g. ion/l. (Fig. 1); these ranges are equivalent to 0–10 and 0–100 p.p.m. Mg^{++} respectively. Under these conditions, no growth of the above organisms occurred in the presence of Ni^{++} , Co^{++} , Zn^{++} , Be^{++} or Fe^{++} . Some growth occurred in the presence of Mn^{++} , but even this metal was somewhat toxic in the higher concentrations (cf. Gwan, 1948), and failed to produce an activation of growth comparable to that produced by Mg^{++} .

Fig. 1

Fig. 2

Figs. 1 and 2. Influence of magnesium and of manganese concentration on the growth of certain Gram-positive and Gram-negative bacteria in a chemically-defined medium (Koser & Rettger, 1919) free from these ions. Growth was measured turbidimetrically with the Spekker photoelectric absorptiometer as previously described (Webb, 1949*b*).

Fig. 1. Effect of magnesium concentration (●—●) and manganese concentration (○—○) on the growth of *B. mycoides*. (○—○), effect of the manganese concentration of the medium on the growth of *B. vulgatus*.

Fig. 2. Effect of magnesium concentration (●—●) and manganese concentration (○—○) on the growth of *A. cloacae*. (○—○), effect of the manganese concentration of the medium on the growth of *Esch. coli commune*.

DISCUSSION

The ability of Mn^{++} to replace Mg^{++} in the formation of the Gram complex and, under certain conditions, in the activation of cell division is in accordance with other findings in biological systems. Thus, as is well known, many enzymes which require Mg^{++} for activation may also be activated by Mn^{++} (e.g. Nilsson, Alm & Burstrom, 1942). However, it appears from the results given in Figs. 1 and 2 that in certain of the Mg^{++} -activated enzyme reactions associated with growth and metabolism the function of magnesium cannot be replaced by

manganese. In this connexion, it is of interest to recall that Quastel & Webley (1942) found that Mn^{++} could not replace Mg^{++} in the activation of the oxidation of acetate by certain bacteria which required vitamin B₁ as a nutrient.

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