Magnesium Transport in Escherichia coli

INTERFERENCE BY MANGANESE WITH MAGNESIUM METABOLISM*

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SUMMARY

Escherichia coli has a specific active transport system for magnesium which can be studied with the radioisotope $^{25}$Mg. The accumulation of $^{25}$Mg is temperature-dependent and inhibited by cyanide, dinitrophenol, and m-chlorophenyl carbonylcyanide hydrzone. The initial rate of uptake of $^{25}$Mg shows typical saturation kinetics with a $K_m = 18 \mu M$ magnesium and a $V_{max} = 0.50 \mu$ mole per min per $10^{12}$ cells at $25^\circ$ in Tris medium. Neither calcium nor strontium competes with, inhibit, or stimulate the transport of $^{25}$Mg. However, magnesium shows complex effects on cellular magnesium metabolism. (a) Manganese appears to be a competitive inhibitor for the magnesium accumulation system with a $K_i = 0.5 \text{ mm}$ manganese in Tris. (b) The addition of high (5 to 10 mm) manganese causes the rapid loss of $^{25}$Mg from previously loaded cells and a net loss of magnesium, as if manganese were displacing internal bound magnesium which then leaves the cells via the magnesium transport system.

Magnesium, the most common intracellular bivalent cation, plays many key intracellular roles including activating many enzymes and stabilizing ribosomes. However, it was only last year that the first reports of a specific active transport system for magnesium were studied. A third laboratory whose published reports (3, 4) and unpublished work argue against the need for a magnesium transport system.

The kinetic parameters of magnesium transport ($K_m$ and $V_{max}$) have been carefully measured with the radioisotope $^{25}$Mg and found to depend on the medium in which they are determined. This was important since Lusk and Kennedy (2) had reported a $K_m$ of 4 $\mu$M magnesium in Tris. (b) The complex interactions of Mn$^{2+}$ with cellular magnesium have been studied. (i) Manganese is a competitive inhibitor of the uptake of magnesium by the cells with a $K_i$ approximately 25 times higher than the $K_m$ for magnesium under comparable conditions. (ii) Once high manganese concentrations accumulate within the cell via the magnesium transport system, they cause the rapid loss of cellular magnesium, presumably by exchanging with ribosome-bound magnesium and raising the intracellular concentration of free magnesium which then leaves the cell by specific transport system(s).

After the first draft of this paper was prepared, we exchanged manuscripts with Nelson and Kennedy (2) who have pursued a very similar line of investigation and with largely similar conclusions. The main differences between the results of the two studies are in the kinetic constants describing the phenomena and, when we have used Mn$^{2+}$ salts to compete with and displace cellular magnesium, Nelson and Kennedy (2) have used Co$^{2+}$.

**EXPERIMENTAL PROCEDURE**

*Bacteria—Several substrains of E. coli B and K-12 were used interchangeably without noticeable effects on results.

*Media—Two basic media were used: a low magnesium (35 $\mu$M Mg$^{2+}$ by atomic absorption spectroscopy) dilute tryptone broth containing 4 g per liter of Difco Bacto-tryptone and 2.5 g per liter of NaCl and a Tris-glucose minimal medium containing 0.12 M Tris-HCl (pH 7.0), 80 $\mu$M NaCl, 20 $\mu$M KCl, 20 $\mu$M NH$_4$Cl, 3 $\mu$M Na$_2$SO$_4$, 0.64 mM KH$_2$PO$_4$, 0.2% glucose, 2 $\mu$M FeCl$_3$, and MgCl$_2$ as desired, generally 1 mM for high magnesium growth conditions.

$^{25}$Mg—$^{25}$Mg ($\lambda = 21.3$ hours) was obtained from the Brookhaven Laboratory Division, Brookhaven National Laboratory, Upton, New York. Since our earlier report (1), the method of producing $^{25}$Mg has been changed by the Brookhaven Laboratory (5) and current specific activities are about 200 $\mu$Ci of $^{25}$Mg-$^{28}$Al per 0.2 mg of Mg$^{2+}$ at time of shipment. This represents a 10-fold improvement and allows us to add as little as 1 $\mu$M radioaactive magnesium and still obtain sufficient incorporation to count with ease.

The general procedures for growth of cells, incorporation of radioactivity, and filtration have been previously described (1, 6-8). Briefly, for influx experiments cells were grown at 37$^\circ$ in nonradioactive growth medium and distributed in small flasks at room temperature. Inhibitors of uptake or nonradioactive
salts were added, followed by the addition of *8Mg. One-milli-
liter samples were filtered through 25-mm Millipore HA filters and washed. The dried filters were glued on planchets and counted in a Nuclear-Chicago low background gas flow counter. For efflux experiments, the cells were allowed to accumulate radioactivity for several generations during growth at 37°, centrifuged, and resuspended in nonradioactive medium. A series of aliquots was distributed at room temperature (in order to reduce the rate of exchange). Samples were filtered and either (a) both the unwashed filter and a dried fraction of the filtrate were counted in the gas flow counter to determine the distribution of radioactivity, or (b) in more recent experiments the filters were washed once with 5 ml of nonradioactive medium and the fraction of radioactivity remaining on the filters was normalized by setting the first filtered sample at 100% (within an experiment replicate samples filtered at the same time varied by less than 10% from their mean value). The advantages of the first procedure are that the actual distribution of radioactivity is measured and that small changes in the filtrate value can be determined accurately when most radioactivity remains in the cells. The advantages of the second procedure are that there are half as many samples to count and that washing removes the 8% or so of the radioactivity which remains on the filters when 1 ml of cell-free radioactive medium is filtered but not washed. Washing the filters increases the resolution when there is little radioactivity left in the cells. *K experiments were identical with the *8Mg experiments except for the replacement of radioactive magnesium with *4K purchased from International Chemical and Nuclear Corporation, Irvine, California.

Cell numbers were determined with a Petroff-Hauser counting chamber and a phase contrast microscope.

An atomic absorption attachment to a Zeiss PMQ II spectrophotometer was used to measure total magnesium in cells grown in different external concentrations of magnesium after centrifugation and washing with water (Fig. 4). CCCP was purchased from Calbiochem, and dinitrophenol from Sigma. Chloramphenicol was the gift of Parke, Davis, and Company. All other chemicals were analytical grade and special “spectral” grade salts were not used.

**RESULTS**

**Uptake of Radioactive Magnesium**—Some general properties of the magnesium transport system in *E. coli* are shown in the experiment in Fig. 1A. Cells were grown in Tris-glucose plus 1 mM magnesium, centrifuged, and resuspended in magnesium-free medium containing the inhibitor of protein synthesis, chloramphenicol. When 10 μM *8Mg was added, the time course of *8Mg uptake showed approximately hyperbolic kinetics, reaching an equilibrium with a little more than 10% of the radioactivity taken up by the cells. Since 1.2 × 10⁸ cells per ml occupy 0.01% (v/v) of the space, this represents 10 + 0.01 = 1000-fold concentration of Mg²⁺. Whether the intracellular magnesium is bound or free is discussed below, but it can be seen in Fig. 1A that the addition of 1 mM nonradioactive magnesium during the course of the uptake experiment results in the loss from the cells of at least 75% of the already accumulated *8Mg. Nonradioactive magnesium (1 mM) added prior to *8Mg competes for the uptake of *8Mg, suggesting that the mag-
The magnesium uptake system can be saturated. Finally, in Fig. 1A, we can see the specificity of the magnesium uptake system of E. coli, since 1 mM manganese, calcium, and strontium do not inhibit \(^{25}\text{Mg}\) uptake. The effect of manganese on magnesium uptake is complex: over the first 10 min there is a small inhibition of uptake, but during the subsequent 50 min there is a small stimulation by manganese of magnesium uptake. This manganese-stimulated uptake of \(^{25}\text{Mg}\) is very reproducible (1) and our tentative explanation for it follows other experiments under "Discussion."

The experiment shown in Fig. 1A was carried out with E. coli K-12 in Tris-glucose medium, whereas our previously reported work (1) was with E. coli B in a tryptone broth with twice the concentrations of NaCl and tryptone as that used in our current experiments. We have never seen significant differences between the K-12 and B strains with regard to \(^{25}\text{Mg}\) metabolism. There are differences associated with medium changes as seen in Figs. 1 and 2. In Fig. 1B, the effects of adding nonradioactive magnesium and manganese on \(^{25}\text{Mg}\) uptake and retention in tryptone broth are shown. When 1.5 mM Mg\(^{2+}\) was added to the cells accumulating \(^{25}\text{Mg}\) in tryptone, there was an immediate cessation of uptake but no loss of previously accumulated \(^{25}\text{Mg}\) as seen in Fig. 1A after the addition of 1 mM Mg\(^{2+}\). Manganese at 5 mM does not stimulate magnesium uptake but is almost as inhibitory as 0.5 mM nonradioactive magnesium.

The effects of nonradioactive magnesium and manganese on the uptake of \(^{25}\text{Mg}\) were carefully measured over a series of concentrations in both Tris-glucose and broth media. The rates of uptake of \(^{25}\text{Mg}\) were followed by collecting samples on filters over 30- to 60-min periods. The initial rate of uptake of \(^{25}\text{Mg}\) was plotted according to Lineweaver and Burk (9). Broth-grown cells were studied either in broth or after centrifugation and resuspension in Tris glucose. Both the apparent \(K_m\) and \(V_{max}\) for \(^{25}\text{Mg}\) uptake are medium-dependent (Fig. 2). The apparent \(K_m\) is 18 \(\mu\)M magnesium in Tris-glucose and 31 \(\mu\)M in tryptone broth. The \(V_{max}\) is 0.56 \(\mu\)moles per min.\(\cdot\)10\(^{12}\) cells in Tris-glucose and 0.20 \(\mu\)moles per min.\(\cdot\)10\(^{12}\) cells in broth. Although values vary slightly from experiment to experiment the direction of the differences is reproducible and the \(K_m\) is higher and the \(V_{max}\) is lower in tryptone broth than in Tris-glucose.

We next addressed the nature of the inhibition of magnesium uptake by manganese seen in Fig. 1 and asked whether this inhibition showed a competitive or noncompetitive relationship with magnesium concentration. As seen in Fig. 3, the inhibition of magnesium uptake by manganese follows classical competitive inhibition kinetics with a \(K_i\) of 0.5 \(\mu\)M manganese in Tris-glucose or 2-fold higher than the \(K_m\) for magnesium. In tryptone broth for which the \(K_m\) for magnesium is 2-fold higher, the inhibition by manganese also appears to be competitive with a \(K_i\) of about 2 \(\mu\)M.

We have also characterized the magnesium transport system as being energy-dependent, inhibited by cyanide, dinitrophenol, and CCCP, and dependent on temperature (1).

Cellular Magnesium Content as Function of External Mg\(^{2+}\)---Although the initial rate of uptake of \(^{25}\text{Mg}\) is dependent on external magnesium (Fig. 2), whether intracellular magnesium varies with extracellular magnesium has been the subject of some contention. Therefore, we measured total cellular Mg\(^{2+}\) by atomic absorption spectroscopy under conditions similar to our
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Mg transport in E. coli was grown in tryptone broth supplemented with added MgCl₂ at 37°C for 3 hours. After centrifuging the cells and washing with water twice, the total cellular magnesium was determined by atomic absorption spectroscopy. The results from two independent experiments are shown. In the first experiment the cells were grown to 1.0 × 10⁹ per ml; in the second experiment the cells grew to 1.4 × 10⁹ per ml and, in the unsupplemented tryptone broth, 70% of the Mg⁺⁺ was taken up by the cells during growth. In the second experiment, samples of cells grown in low magnesium broth were exposed for 30 min at 25°C to higher magnesium concentrations ("step up") prior to centrifugation (Δ).

In both experiments, the Mg⁺⁺ content of E. coli varies about 1 part in 1000 of the nonradioactive 100 mM magnesium. Some experiments in E. coli are constitutive and others repressible (12, 13). Cells were grown in low and high magnesium, centrifuged, washed, and resuspended in low magnesium broth, and their ability to concentrate ²⁸Mg was measured. As seen in Fig. 5, ²⁸Mg uptake is independent of the growth medium, showing that the transport system is constitutive.

Constitutivity of Magnesium Transport System—Concentrations of magnesium above 0.15 mM are growth inhibitory in our dilute tryptone broth, but this is already 6 times the total internal concentration from Fig. 4 (25 µmoles in 10¹² cells which occupy about 1 ml of space and are 75 to 80% water). Since the cells can be grown in higher concentrations than are needed intracellularly, we could next ask whether the magnesium transport system is synthesized constitutively during growth in high magnesium or whether the magnesium transport system is repressible and only synthesized under conditions in which it is needed to raise the intracellular magnesium to 25 mM. Some carbohydrate transport systems in E. coli are constitutive and others repressible (12, 13). Cells were grown in low and high magnesium, centrifuged, washed, and resuspended in low magnesium broth, and their ability to concentrate ²⁸Mg was measured. As seen in Fig. 5, ²⁸Mg uptake is independent of the growth medium, showing that the transport system is constitutive.

Constitutivity of the magnesium transport system. Cells were grown to 5.8 × 10⁹ per ml in tryptone broth supplemented with added MgCl₂ 100 µM. Chloramphenicol were added and the cells were harvested by centrifugation, washed twice in low magnesium tryptone broth, and again resuspended in low magnesium (35 µM) tryptone broth. ²⁸Mg was added and uptake was followed by filtration at 24°C. Chloramphenicol was present throughout the centrifugations and the exposure to ²⁸Mg. The uptake of 10% of the ²⁸Mg corresponds to 6 micromoles per 10¹² cells.

In agreement with previously reported experiments (10, 11), the Mg⁺⁺ content of E. coli varies by less than a factor of 2 (15 to 28 µmoles/10¹² cells) when the external magnesium concentration is varied by a factor of 2500 (Fig. 4). Data from two experiments are shown in Fig. 4 and the cellular Mg⁺⁺ content shows the same less than a 2-fold increase when extracellular magnesium during growth was increased from 0.1 mM to 0.1 mM in both experiments. In the second experiment two additional points can be seen. (a) Cells grown in broth containing 35 µM Mg⁺⁺ to concentrations as high as 1.5 × 10⁻¹⁰ per ml have a lower Mg⁺⁺ content per cell both because the cells become smaller toward the end of growth and because of the onset of magnesium starvation with more than 70% of the available magnesium in the cells. (b) When such low magnesium cells are exposed to "step up" conditions of higher concentrations of magnesium for 30 min at room temperature (the conditions of the radioactive experiments in Figs. 1 to 3), the intracellular Mg⁺⁺ does not change as a function of extracellular Mg⁺⁺.

Experiments with ²⁸Mg Efflux—Ideally, if the magnesium transport system is mediating two-compartment exchange kinetics between osmotically free external and internal magnesium pools and the internal magnesium concentration is independent of the external concentration, we could monitor the system either by measuring (a) uptake or accumulation or by measuring (b) efflux or turnover exchange, with completely consistent results and kinetic parameters. The limits of this simplistic approach are seen in Figs. 6 and 7 where the specificity of the efflux of ²⁸Mg was tested to compare with the experiments in Figs. 1–3. The results of these two types of experiments are rather similar: calcium neither affects the uptake of magnesium nor does it influence the loss of radioactive magnesium from the cells (Fig. 6). Increasing the magnesium from 35 µM (near the Kₘ for uptake) to 10 mM increases the rate of efflux about a factor of 2 (Fig. 6), as would be expected since the rate of magnesium uptake at 10 mM magnesium should be about twice the rate at 35 µM magnesium. However, 5 mM Mn⁺⁺, which is a poorer competitor than Mg⁺⁺ for ²⁸Mg uptake, causes a rapid
Over additional points are apparent. (a) Increasing the external Mg\(^2+\), which is in satisfactory accord with the Ri for Mnl+ in estimation of a half-saturation concentration for Mg efflux in tryptone broth with 0.1 mM added Mg\(^2+\) as the rate constant k in the equation samples were filtered.

and then determining the rate of loss of BMg for the exponential loss region of the curve (generally the initial rate after MnCl\(_2\) was added) as the rate constant k in the equation $\frac{\Delta \text{M}_{\text{G}}}{\Delta t} = e^{-kt}$. The results, shown in Fig. 7, allow the estimation of a half-saturation concentration for \(28\text{Mg}\) efflux in tryptone broth with 0.1 mM added Mg\(^2+\) of about 2 to 5 mM Mn\(^{2+}\), which is in satisfactory accord with the $K_i$ for Mn\(^{2+}\) in broth as a competitive inhibitor of \(28\text{Mg}\) uptake. Two additional points are apparent. (a) Increasing the external Mg\(^2+\) over a range of concentrations 1000-fold higher than the $K_a$ for the \(28\text{Mg}\) accumulation system causes an increasingly rapid rate of loss of \(28\text{Mg}\) without any apparent maximum rate or plateau. (b) Even growth inhibitory concentrations of Mg\(^2+\) (0.25 mM) do not cause a rapid loss of \(28\text{Mg}\) from the cells as do growth inhibitory concentrations of Mn\(^{2+}\) (10 mM).

We have not directly determined whether manganese is a substrate for as well as a competitive inhibitor of the magnesium accumulation system. Nevertheless, as shown in Figs. 8 and 9, the Mn\(^{2+}\)-induced loss of \(28\text{Mg}\) is energy-dependent and Mg\(^2+\) protects the cells against this effect of Mn\(^{2+}\) in a way consistent with the hypothesis that Mn\(^{2+}\) is entering the cell via the magnesium transport system. Cellular exchange of radioactive \(28\text{Mg}\) for nonradioactive magnesium is inhibited by energy poisons such as cyanide, dinitrophenol, and adro (1, 2, 7). As can be seen in Fig. 8 the addition of dinitrophenol or CCCP by itself does not cause \(28\text{Mg}\) leakage, but rather promotes the retention of cellular radioactivity. Similarly, the manganese-induced loss of \(28\text{Mg}\) is also inhibited by energy poisons including cyanide, dinitrophenol (Fig. 8A), and CCCP (Fig. 8B). This suggests that the manganese must enter the cell via an energy-dependent system or that intracellular manganese-magnesium exchange is energy-dependent (or both).

Formaldehyde has been reported to prevent loss of small molecules from the cells by sealing or “tanning” the surface transport carriers (14). The effects of adding formaldehyde on the manganese-induced loss of radioactive magnesium are seen in Fig. 9A. Formaldehyde treatment completely eliminates manganese-induced magnesium loss. In order to compare transport-mediated loss of \(28\text{Mg}\) with passive leakage, sufficient toluene (1% v/v) was added to cause passive loss of \(28\text{Mg}\) at a rate comparable to the manganese-induced loss. Formaldehyde does slow the rate of loss of \(28\text{Mg}\) in toluene-treated cells, but it does not eliminate the loss as with manganese-treated cells.

To pursue still further our working hypothesis that the manganese is entering the cell via a transport system and then causing the exchange loss of \(28\text{Mg}\), we addressed the question as to whether transport system(s) might be involved. If the manganese enters the cells via the normal magnesium transport system, then added nonradioactive magnesium should competitively inhibit the manganese-stimulated loss of \(28\text{Mg}\), as is in fact seen in Fig. 9B. Although the degree of protection is variable depending on the exact concentrations of magnesium and manganese used,

\[ K_i = e^{-kt} \]
Specificty of Effects of Manganese on Magnesium Metabolism—

We have just described effects of manganese on both the uptake and loss of $^{28}\text{Mg}$ from \textit{E. coli}. In order to know at what level Mn$^{2+}$ is acting, it is essential to know whether high Mn$^{2+}$ only affects cellular Mg$^{2+}$ metabolism or whether other systems are also influenced. Since potassium is the principal intracellular cation, and since the control and exchange of intracellular potassium have been thoroughly studied (16, 17), we systematically looked for effects of manganese on the uptake and loss of $^{42}\text{K}$ so as to be able to distinguish specific effects on the magnesium transport system from more general effects on cell permeability and osmotic regulation. Typical results with $^{42}\text{K}$-labeled cells in dilute tryptone broth are seen in Fig. 10. Whereas 1 mM Mn$^{2+}$ has a discernible effect on the uptake of $^{28}\text{Mg}$ and 10 mM Mn$^{2+}$ inhibits magnesium uptake more than 90\% (Figs. 1 and 3), there is no effect of 1 mM Mn$^{2+}$ on $^{42}\text{K}$ uptake by \textit{E. coli} and 10 mM Mn$^{2+}$ (which is growth inhibitory) slightly stimulates $^{42}\text{K}$ uptake over the period of 1 hour (Fig. 10A). Even 50 mM Mn$^{2+}$ is stimulatory for $^{42}\text{K}$ uptake for the first 5 to 10 min of exposure. Therefore, we can conclude that Mn$^{2+}$ does not inhibit $^{42}\text{K}$ uptake under the conditions in which $^{28}\text{Mg}$ uptake is inhibited. Manganese at 10 mM causes a slightly accelerated rate of loss.
The manganese-induced loss of radioactive potassium does not result in a net loss of potassium since uptake is also stimulated (Fig. 10A)—it is the rate of turnover or exchange of intracellular for extracellular potassium which is increased by high manganese. The manganese-stimulated loss of \(^{42}\text{K}\) is inhibited by energy poisons such as CCCP (Fig. 10B). CCCP causes a very rapid loss of about 30% of the cellular \(^{42}\text{K}\) followed by a slower rate of loss of the remaining \(^{42}\text{K}\). It is during this slower phase of \(^{42}\text{K}\) loss or turnover that neither 10 mM K\(^+\) nor Mn\(^{2+}\) increases the rate of loss of \(^{42}\text{K}\) (Fig. 10B).

Following the logic used in the experiment shown in Fig. 9B, that Mg\(^{2+}\) might compete for Mn\(^{2+}\) uptake via the normal magnesium transport system and thereby prevent Mn\(^{2+}\)-induced loss of radioisotope from the cells, we asked whether Mg\(^{2+}\) could prevent the manganese-stimulated loss of \(^{42}\text{K}\). As seen in Fig. 11, high magnesium does protect the cells against manganese-induced loss of \(^{42}\text{K}\), and furthermore this effect of magnesium is specific in that high magnesium does not affect the rate of \(^{42}\text{K}\) loss (turnover) stimulated by 25 mM nonradioactive K\(^+\).

There is another side to the question of specificity. Do other transition elements have similar effects on magnesium metabolism to that of manganese? Apparently not; under the conditions of these experiments and when marginally inhibitory concentrations are added, Co\(^{2+}\), Fe\(^{2+}\), Ni\(^{2+}\), and Cr\(^{3+}\) do not

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**Figure 10.** Effects of high manganese concentrations on cellular potassium (\(^{42}\text{K}\)) metabolism. \(A\), uptake in tryptone broth. As in Fig. 1B, except that \(^{42}\text{K}\) (0.1 \(\mu\text{Ci}\) per ml; 1.5 \(\mu\text{m}\)) was added 2 min after the addition of MnCl\(_2\). \(B\), efflux or loss. As in Fig. 6, except that the cells were grown with radioactive \(^{42}\text{K}\). (turnover) of previously loaded \(^{42}\text{K}\) from the cells (Fig. 10B).

**Figure 11.** Protection by magnesium of manganese-induced loss of \(^{42}\text{K}\). As in Fig. 10B, except that 5 mM MgCl\(_2\) was added where indicated 30 sec prior to the addition of 10 mM MnCl\(_2\) or 25 mM KCl.
cause accelerated loss of previously loaded $^{25} \text{Mg}$.\(^7\) At higher concentrations, effects are seen and with very different conditions, Nelson and Kennedy\(^8\) are observing effects similar to ours but with Ce\(^{4+}\) instead of Mn\(^{2+}\).

**DISCUSSION**

*E. coli* has a highly specific magnesium accumulation system with the usual criteria for active transport. The uptake of $^{25} \text{Mg}$ is temperature-dependent (1, 2) and inhibited by a wide range of energy poisons (1, 2). The initial rate of $^{25} \text{Mg}$ accumulation shows classical saturation kinetics with a $K_m$ of 31 $\mu$M and a $V_{max}$ of 0.2 pmole per min per 10\(^8\) cells in dilute broth. The kinetic parameters for magnesium accumulation are medium-dependent and in Tris-glucose the $K_m$ is 18 $\mu$M and the $V_{max}$ is 0.56 pmole per min per $10^8$ cells (Fig. 2). Manganese is a competitive inhibitor of $^{25} \text{Mg}$ accumulation with a $K_i$ of about 2 $\mu$M in broth and 0.5 $\mu$M in Tris-glucose (Fig. 3).

Manganese efflux from *E. coli* is also temperature- and energy-dependent (1, 2, 7; Fig. 8), suggesting that exit as well as uptake is carrier-mediated. The rates of uptake and loss of magnesium by the cells vary with external concentration so as to maintain a relatively constant cellular magnesium content (Fig. 4) regardless of the external concentration. For example, at 1 mM external magnesium in broth, the rate of uptake is near the $V_{max}$ of 0.2 pmole per min per $10^8$ cells. The rate of turnover or efflux of radioactive $^{25} \text{Mg}$ is about 0.01 pmol per min. The second-order rate constant of 25 pmol/10\(^8\) cells per sec corresponds to a 0.25 pmole per min per $10^8$ cells. In Tris-glucose medium the cellular magnesium content is about the same (10) but the maximum rate of magnesium uptake (Fig. 2) and turnover\(^2\) is higher.

**Cellular Magnesium**—What fraction of the cellular magnesium is bound or osmotically free is not measured in our experiments, but this question is important to our understanding of the magnesium accumulation system and in particular for an understanding of the effect of manganese on magnesium metabolism. Most of the intracellular magnesium appears to be bound to ribosomes and other intracellular polyanions (see Reference 3 for a discussion of the evidence).

**Effects of Manganese on Magnesium Metabolism**—The interactions of manganese with cellular magnesium are clearly complex and, in addition to its function as a competitive inhibitor of uptake (Fig. 3), the displacement of $^{25} \text{Mg}$ from the cell by manganese must be explained (Figs. 6 to 9). Our current hypothesis for these effects has been developed under "Results" and need be brought together only briefly here. Manganese is not only a competitive inhibitor of magnesium accumulation but appears to enter the cells via this accumulation system (this has not been directly measured in our experiments\(^3\)). Once within the cells, manganese freely exchanges with ribosomal bound magnesium (3, 20, 21), increasing the free intracellular concentration of magnesium which then leaves the cells via the magnesium transport system. At inhibitory concentrations of manganese (5 to 10 $\mu$M), the result is a net loss of cellular magnesium without striking changes in cellular permeability toward other "free" intracellular cations such as potassium (Fig. 10). With increasingly higher concentrations of manganese, magnesium involved in membrane stability (22, 23) is also displaced, leading to a more general breakdown of the cellular permeability barriers (Figs. 10 and 11). This model for the action of manganese on *E. coli* also accounts for the sigmoidal course of $^{25} \text{Mg}$ uptake seen in the presence of 1 mM Mn\(^{2+}\) (Fig. 1A and Reference 1); first the inhibition of Mg\(^{2+}\) uptake is due to direct competition for the transport carrier. Then following net magnesium loss, a type of feedback control results in an increasing rate of magnesium uptake as the cell attempts to maintain a constant cellular magnesium content (10, 24). Similar explanations would account for the increased uptake of $^{25} \text{K}$ in 10 mM Mn\(^{2+}\) (Fig. 10A). Control over the cellular rate of sulfate active transport (25) and of sugar uptake in subcellular membrane vesicles (26) has also been described.

The kinetic analysis of magnesium accumulation leads to the prediction of a class of bacterial mutants with an altered magnesium carrier system with a lower affinity for manganese. These will be manganese-resistant in the presence of high magnesium concentrations. We have isolated a series of manganese-resistant magnesium-dependent mutants of *E. coli* which appear to fulfill the requirements for carrier mutants with a higher $K_i$ for manganese in the magnesium transport system. The growth, genetics, and magnesium transport characteristics of these strains will be described shortly.\(^9\)

**REFERENCES**

1. Silver, S., Proc. Nat. Acad. Sci. U. S. A., 63, 704 (1969).
2. Lusk, J. E., and Kennedy, E. P., J. Biol. Chem., 244, 1653 (1969).
3. Hurwitz, C., and Rosano, C. L., J. Biol. Chem., 242, 3719 (1967).
4. Rosano, C. L., and Hurwitz, C., Biochim. Biophys. Res. Commun., 37, 677 (1969).
5. Alberts, D. E., and Harris, W. R., Phys. Rev., 185, 1495 (1969).
6. Silver, S., and Wenden, L., J. Bacteriol., 93, 560 (1967).
7. Silver, S., Levine, E., and Spirigel, P. M., J. Virol., 2, 763 (1968).
8. Silver, S., and Levine, E., J. Bacteriol., 96, 338 (1968).
9. Lineweaver, H., and Buek, D., J. Amer. Chem. Soc., 86, 655 (1934).
10. Lusk, J. E., Williams, R. J. P., and Kennedy, E. P., J. Biol. Chem., 243, 2018 (1969).
11. Tempest, D. W., Diina, J. W., and Meeks, J. L., J. Gen. Microbiol., 49, 159 (1967).
12. Ganesan, A. K., and Rotman, B., J. Mol. Biol., 16, 42 (1965).
13. Simoni, K. J., Levinthal, M., Kündig, F. D., Kundig, W., Anderson, B., Hartman, P. E., and Rosenman, S., Proc. Nat. Acad. Sci. U. S. A., 58, 165 (1965).
14. Koch, A. L., Biochim. Biophys. Acta, 78, 177 (1964).
15. Winkler, H. H., and Wilson, T. H., J. Bacteriol., 98, 2120 (1966).
16. Epstein, W., and Schulz, S. G., J. Gen. Physiol., 49, 221 (1965).
17. Epstein, W., and Schulz, S. G., J. Gen. Physiol., 49, 469 (1965).
18. Silver, S., and Krulovic, M. L., Biochim. Biophys. Res. Commun., 34, 940 (1969).
19. Silver, S., Johnseine, P., and King, E., J. Bacteriol., 104, 1299 (1970).
20. KRodgers, A., Biochim. J., 39, 448 (1964).
21. Hubbard, B., Miale, S. H., Priscoe, A. R., Walker, I. O., and Richardson, R. E., J. Mol. Biol., 26, 389 (1967).
22. Silver, S., and Levine, E., Biochim. Biophys. Res. Commun., 31, 743 (1968).
23. Fitz, A., and Prantton, D., J. Bacteriol., 96, 1209 (1969).
24. Gunther, T., and Dorn, F., Z. Naturforsch., 2b, 713 (1969).
25. Dreyfuss, Y., and Parker, A. B., J. Bacteriol., 91, 2275 (1966).
26. Kadis, H. H., Proc. Nat. Acad. Sci. U. S. A., 64, 724 (1969).

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