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# Major intracellular cations and growth control: Correspondence among magnesium content, protein synthesis, and the onset of DNA synthesis in BALB/c3T3 cells

(translational control/sodium/potassium/calcium)

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**ABSTRACT** **Omission of $Ca^{2+}$ from the medium of confluent BALB/c3T3 cells for a period of 17 hr causes a large decrease in the number of cells synthesizing DNA. This effect is reversed by raising the $Mg^{2+}$ concentration of the medium to 20 mM. However, if the [$Mg^{2+}$] is greater than 20 mM ("ultrahigh" $Mg^{2+}$), there is again a decrease in the number of cells synthesizing DNA. The synthesis of protein has a similar dependence on $Mg^{2+}$ concentration in $Ca^{2+}$-deficient medium, but it responds within 45 min of the shift in cation concentrations rather than the 10 hr that is required for the change in DNA synthesis to become apparent. Cells in the ultrahigh $Mg^{2+}$ concentrations that are at first inhibitory to protein synthesis later return to maximal protein synthesis. This delayed increase in protein synthesis is reflected in a delayed increase in DNA synthesis. Intracellular concentrations of $Mg^{2+}$ in $Ca^{2+}$-deficient media increase in proportion to extracellular $Mg^{2+}$ concentrations. Cells in medium with 30 mM $Mg^{2+}$ have a high intracellular content of $Mg^{2+}$ at 3 hr but have decreased their intracellular content by 17 hr, a time at which protein synthesis has been restored to normal. Intracellular $Na^+$ and $K^+$ concentrations also change in $Ca^{2+}$-deficient medium, but independent variation of these ions shows that protein synthesis is relatively insensitive to their concentration. Intracellular $Ca^{2+}$ remains fairly constant under all these conditions. The rate of protein synthesis of intact cells changes as a function of intracellular $Mg^{2+}$ content in a manner very similar to that which has been reported for cell-free systems. We conclude that protein synthesis is very sensitive to small changes in intracellular [$Mg^{2+}$] within physiological range and that the onset of DNA synthesis is dependent on the rate of protein synthesis. Regulation of the availability of $Mg^{2+}$ within the cell therefore presents a plausible mechanism for growth control.**

Animal cells respond to individual external effectors such as hormones or proteolytic enzymes by accelerating a stereotyped group of reactions associated with transport (1), intermediary metabolism (2, 3), and macromolecular synthesis (4–6). Some of the early responses are independent of one another and of macromolecular synthesis (1). The aggregate of these reactions has been designated the coordinate response (7, 8), to distinguish it conceptually from the pleiotypic response (9) because the former embraces the differentiated functions of the cell whereas the latter explicitly excludes them (10). The diverse elements of the coordinate response can be modulated by varying the $Mg^{2+}$ concentration of the medium, especially in cells made permeable to $Mg^{2+}$ by withdrawing extracellular $Ca^{2+}$ (11–13). We have proposed that $Mg^{2+}$ plays an important role in mediating the coordinate response through its regulation of transphosphorylation and other $Mg^{2+}$-dependent reactions (7, 8). This proposal is supported by the finding that insulin and serum increase the intracellular concentrations of $Mg^{2+}$ in chicken cells and BALB/c3T3 cells in culture (14, 15). Recently we reported that the stimulation of onset of DNA synthesis in quiescent BALB/c3T3 cells by high concentrations of $Mg^{2+}$ in $Ca^{2+}$-deficient medium reaches a maximum at 15–20 mM $Mg^{2+}$; higher concentrations of $Mg^{2+}$ inhibit the onset of DNA synthesis, and still higher concentrations kill the cells (13).

At least 10 hr are required after the addition of serum to BALB/c3T3 cells before an increase can be detected in the number of cells making DNA (13). We set out to determine whether any of the earlier responses have the same profile of sensitivity to $Mg^{2+}$ as does the onset of DNA synthesis and have reported that the uptake of uridine was not inhibited by those high concentrations of $Mg^{2+}$ that inhibit the onset of DNA synthesis (12). We show here that relatively small changes in the cellular content of $Mg^{2+}$ are associated with marked alterations in the rate of protein synthesis and that the very high concentrations of $Mg^{2+}$ that inhibit the onset of DNA synthesis after 10 hr of treatment also inhibit protein synthesis but do so in less than 1 hr. We discuss the possible role of intracellular $Mg^{2+}$ in regulating protein synthesis and thereby affecting the onset of DNA synthesis.

## MATERIALS AND METHODS

**Cell Culture and Labeling.** BALB/c3T3 cells were maintained in Dulbecco's modified Eagle's medium (16) with 10% calf serum as described (13). Except where noted, 60-mm polystyrene tissue culture dishes were used. After cultures had become confluent they were switched to modified Eagle's medium with 1% serum before use in experiments. The calf serum used during the experiments was extensively dialyzed against 150 mM NaCl. Modified Eagle's medium was prepared without $Ca^{2+}$ and $Mg^{2+}$, and in some cases without the other salts, which were added later in appropriate concentrations. The maintenance medium was replaced by modified Eagle's medium with 1% calf serum ("turn-down medium") about 18 hr before the start of an experiment.

Labeling with radioactive isotopes was done in medium of the same composition as used during the course of the experiment. Because of the high leucine content of the medium (0.8 mM) and the various additions made for the experiments, particular care was taken to have all labeling with [$^3$H]leucine (original specific activity 51.6 Ci/mmol; 1 Ci = $3.7 \times 10^{10}$ becquerels) done with medium of the same specific activity within a given experiment. Labeling with [$^3$H]thymidine was for 1 hr with 1 $\mu$Ci of [$^3$H]thymidine per ml (specific activity 20 Ci/mmol). The procedures for determining radioactivity and protein content were as described (14, 17).

**Other Procedures.** The procedures for measuring the cellular content of the four major cations by atomic absorption spectrophotometry were as reported (17). Briefly, cultures were washed five times with 10 ml per wash of $CO_2$-free 0.25 M

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sucrose solution, ≈ pH 7.0. They were then exposed to a carbonated sucrose solution, pH 4.0, to displace the externally bound cations. In the experiment of Fig. 6, in which only the $Mg^{2+}$ and $K^+$ contents of the cells were measured, the cells were washed with 150 mM NaCl plus 1 mM $CaCl_2$. Washed cells were scraped from the dish and suspended in distilled water. All samples for atomic absorption spectrophotometry contained 15 mM $La^{3+}$, 4 mM $Cs^+$, and 100 mM HCl to minimize chemical and ionization interferences.

## RESULTS

**$Mg^{2+}$ Effects on the Incorporation of Leucine and Thymidine.** The omission of $Ca^{2+}$ from the medium resulted in a sharp decrease in the rate of leucine incorporation measured 3–5 hr later (Fig. 1*a*). A sharp increase in leucine incorporation in $Ca^{2+}$-deficient medium was produced by raising the $Mg^{2+}$ concentration. Leucine incorporation leveled off when $Mg^{2+}$ was raised above 5 mM and decreased when the $Mg^{2+}$ content of the medium exceeded 20 mM. The rate of thymidine incorporation when measured after 17 hr in the same media increased with $Mg^{2+}$ content up to 20 mM and declined with higher concentrations. The rates of leucine and thymidine incorporation in the $Ca^{2+}$-deficient medium reached levels at least as high in supranormal $Mg^{2+}$ as in medium with physiological concentrations of both ions. Total protein at 17 hr increased in the $Ca^{2+}$-deficient medium with increases in [$Mg^{2+}$] up to 25 mM (Fig. 1*b*).

The discrepancy between optimum concentrations for leucine incorporation at 3–5 hr on the one hand and thymidine incorporation and total recovered protein at 17–18 hr on the other raised the possibility that the rate of leucine incorporation changes with time. Fig. 2 shows the time course of leucine incorporation for 2 hr after culturing cells in $Ca^{2+}$-deficient media with varying [$Mg^{2+}$]. In low [$Mg^{2+}$], the rates of leucine incorporation declined rapidly and reached a minimum value between 1 and 2 hr. In 5 mM $Mg^{2+}$, the rate of leucine incorporation increased during the 2-hr period, as it did to a lesser extent in 15 mM $Mg^{2+}$. In 40 mM $Mg^{2+}$ however, leucine incorporation rapidly declined during the 2-hr period.

A longer term experiment with varying [$Mg^{2+}$] in $Ca^{2+}$-deficient medium (Fig. 3) shows that leucine incorporation of cultures in 15 mM $Mg^{2+}$ continued to increase until 7 hr when it surpassed the rate of incorporation of cultures in 5 mM $Mg^{2+}$. The rates of leucine incorporation in cultures with the lower concentrations of $Mg^{2+}$ in $Ca^{2+}$-deficient medium remained

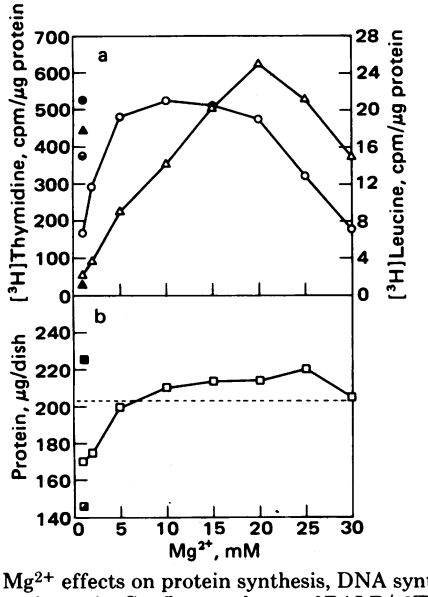


FIG. 1. $Mg^{2+}$ effects on protein synthesis, DNA synthesis, and accumulation of protein. Confluent cultures of BALB/c3T3 cells that had been "turned down" overnight in modified Eagle's medium with 1% serum were washed twice in medium without $Ca^{2+}$ and $Mg^{2+}$. Media containing 0.02 mM $Ca^{2+}$ with varying concentrations of $Mg^{2+}$ and 10% dialyzed calf serum were added to the cultures (open symbols). One group of control cultures was in medium with 1.7 mM $Ca^{2+}$ and 1.0 mM $Mg^{2+}$ with 10% dialyzed serum (filled symbols) and another group was in the used "turn-down" medium with 1% serum (half-filled symbols). Some of the cultures were incubated for 3 hr and switched to medium of the same composition containing 2 μCi of [$^3$H]leucine per ml for 2 hr. The remaining cultures were incubated for 17 hr and labeled in the appropriate medium for 1 hr with 1 μCi of [$^3$H]thymidine per ml. The cultures were then processed to measure radioactivity and protein content. (*a*) ○, ◒, and ●: [$^3$H]Leucine incorporation (3 hr); △, ▲, and ▲: [$^3$H]thymidine incorporation (17 hr). (*b*) □, ◪, and ■: Total protein (17 hr). The broken line in Fig. 1*b* represents protein content of the cultures at 0 hr.

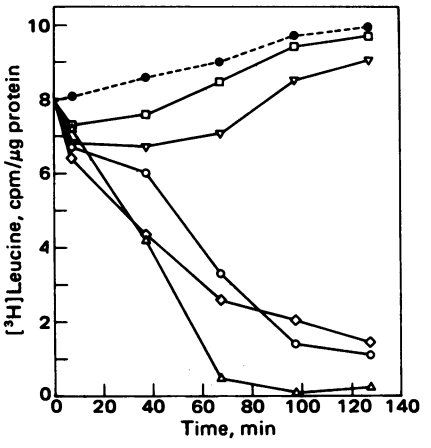


FIG. 2. Early kinetics of $Mg^{2+}$ effects on protein synthesis. "Turned-down" confluent cultures were washed and switched to media of the appropriate cation concentrations with 10% dialyzed serum. They were incubated at 37°C, labeled in the incubation medium for 15 min with 4 μCi of [$^3$H]leucine per ml at various times up to 2 hr, and processed for scintillation counting and protein determination. Open symbols, 0.02 mM $Ca^{2+}$; filled symbols, 1.7 mM $Ca^{2+}$. △, 0.015 mM $Mg^{2+}$; ○ and ●, 1.0 mM $Mg^{2+}$; □, 5.0 mM $Mg^{2+}$; ▽, 15.0 mM $Mg^{2+}$; ◇, 40 mM $Mg^{2+}$. Symbols are plotted at midpoint of 15-min labeling period.

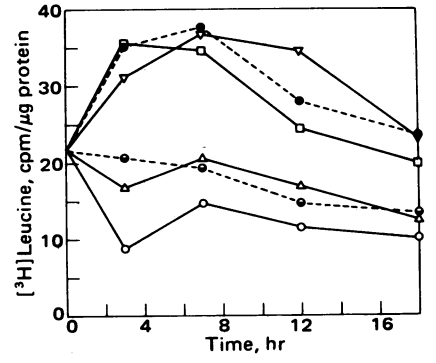


FIG. 3. Extended time course of $Mg^{2+}$ effects on protein synthesis. Confluent cultures were washed and incubated in media with varying concentrations of $Ca^{2+}$ and $Mg^{2+}$ with 10% dialyzed calf serum or in physiological medium with 1% dialyzed calf serum. At the indicated intervals they were labeled for 1 hr with 2 μCi of [$^3$H]leucine per ml and processed for scintillation counting and protein determination. Open symbols, 0.02 mM $Ca^{2+}$ plus 10% dialyzed serum; filled symbols, 1.7 mM $Ca^{2+}$ plus 10% dialyzed serum; half-filled symbols, 1.7 mM $Ca^{2+}$ plus 1% serum. ○, ◒, and ●, 1.0 mM $Mg^{2+}$; △, 2.0 mM $Mg^{2+}$; □, 5.0 mM $Mg^{2+}$; ▽, 15 mM $Mg^{2+}$.

low. The stimulatory effect on protein synthesis of serum in $Ca^{2+}$-sufficient medium was also evident.

A still longer term experiment, which includes ultrahigh [$Mg^{2+}$], shows that cultures in 35 mM $Mg^{2+}$ with low $Ca^{2+}$, which had a decreased rate of leucine incorporation at 5 hr, recovered from this inhibition and were restored to a high rate of incorporation at 17 hr (Fig. 4*a*). The onset of DNA synthesis and increase in total protein were also delayed in these cultures for several hours (Fig. 4 *b* and *c*), suggesting a connection between the three processes. It should be noted however that the rate of leucine incorporation in $Ca^{2+}$-deficient medium with 1 mM $Mg^{2+}$ and 10% serum was consistently lower than that in the $Ca^{2+}$-sufficient medium with only 1% serum, yet the rates of DNA synthesis and accumulation of total protein were higher in the former beginning at 22 hr. This suggests that serum not only stimulates protein synthesis but inhibits protein degradation and that the latter plays an important role in determining the rate of accumulation of protein and the onset of DNA synthesis in these cells. Preliminary results indicate that the rate of protein degradation is unaffected by $Mg^{2+}$ concentration but is inhibited by serum.

**Cation Content of the Cells.** The delay of several hours in the increase of leucine incorporation in the presence of 35 mM $Mg^{2+}$ suggested that high concentrations of $Mg^{2+}$ inhibitory to protein synthesis were present in the cells at 3 hr but were decreased to concentrations more favorable to protein synthesis by 17 hr. Measurement of cellular cations of cultures in various external [$Mg^{2+}$] shows that this is so (Fig. 5). Cultures in 30 mM $Mg^{2+}$ and low $Ca^{2+}$ had a high content of $Mg^{2+}$ and a low rate of leucine incorporation at 3 hr but a lower content of $Mg^{2+}$ and a much higher rate of leucine incorporation at 17 hr. In 50 mM $Mg^{2+}$ however, the intracellular $Mg^{2+}$ content, which was very high at 3 hr, was even higher at 17 hr. Protein synthesis approached zero at both times and the cells eventually died.

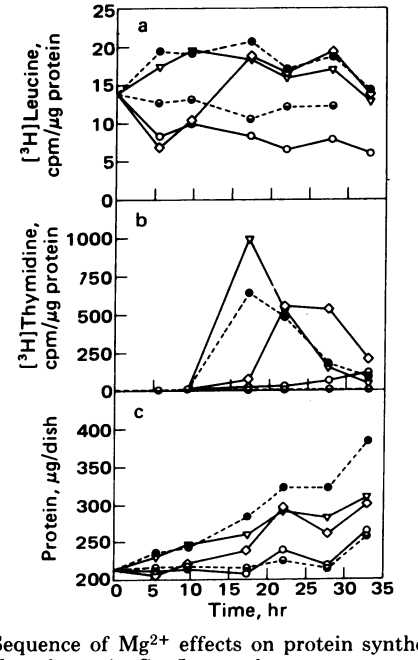


FIG. 4. Sequence of $Mg^{2+}$ effects on protein synthesis, DNA synthesis, and total protein. Confluent cultures were treated as in Fig. 2 but a higher range of concentrations of $Mg^{2+}$ was used, the experiment was extended up to 33 hr, and [$^3$H]thymidine incorporation was measured (b), in addition to measurement of [$^3$H]leucine incorporation (a) and total protein (c). Open symbols, 0.02 mM $Ca^{2+}$ plus 10% dialyzed serum; filled symbols, 1.7 mM $Ca^{2+}$ plus 10% dialyzed serum; half-filled symbols, 1.7 mM $Ca^{2+}$ plus 1% serum. ○, ◒, and ●, 1.0 mM $Mg^{2+}$; ▽, 20 mM $Mg^{2+}$; ◇, 35 mM $Mg^{2+}$.

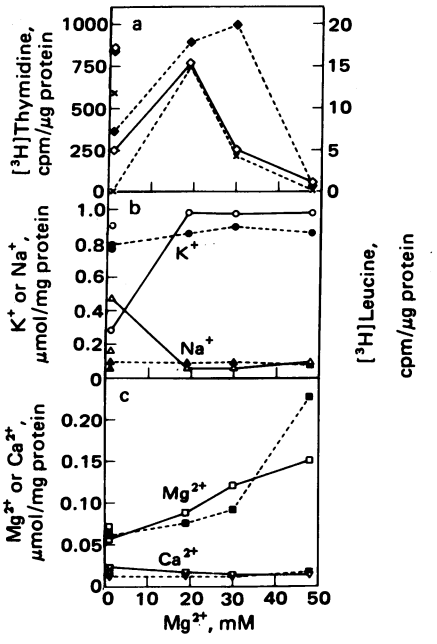


FIG. 5. Intracellular concentrations of four major cations in cultures maintained in varying concentrations of $Ca^{2+}$ and $Mg^{2+}$. Turned-off confluent cultures in 100-mm petri dishes were washed and incubated in 10% dialyzed serum with 1.7 mM $Ca^{2+}$ and 1.0 mM $Mg^{2+}$ (isolated points) or with 0.02 mM $Ca^{2+}$ and 1.0–48 mM $Mg^{2+}$ (points connected by lines). At 3 hr some of the cultures were labeled with [$^3$H]leucine or processed for measurement of intracellular cation content by atomic absorption spectrophotometry. At 17 hr the remaining cultures were similarly processed and an additional group was labeled with [$^3$H]thymidine. Open symbols or continuous line or both, 3 hr; filled symbols or broken line or both, 17 hr. (*a*) ◇ and ◆, [$^3$H]leucine; ×, [$^3$H]thymidine. (*b*) ○ and ●, $K^+$; △ and ▲, $Na^+$. (*c*) □ and ■, $Mg^{2+}$; ▽ and ▼, $Ca^{2+}$.

Removal of $Ca^{2+}$ from medium containing a physiological concentration of $Mg^{2+}$ (≈1 mM) caused a sharp decrease in cellular $K^+$ and an increase in cellular $Na^+$ at 3 hr, but the content of both monovalent cations returned to near normal levels at 17 hr (Fig. 5). The early changes in cellular $K^+$ and $Na^+$ did not occur when higher concentrations of $Mg^{2+}$ were present in the $Ca^{2+}$-deficient medium. Paradoxically, the intracellular content of $Ca^{2+}$ was not decreased when $Ca^{2+}$ was omitted from the medium; indeed it was slightly increased at 3 hr in the presence of physiological [$Mg^{2+}$] (Fig. 5).

Because $K^+$ is required for protein synthesis, the early decrease in cellular $K^+$ could have caused the low rate of protein synthesis in $Ca^{2+}$-deficient medium with physiological [$Mg^{2+}$]. However, the continued inhibition of protein synthesis in these cultures at 17 hr, after $K^+$ had returned to near physiological concentrations (Fig. 5), made this unlikely. To pursue the question further, we added $K^+$ in increasing concentrations up to 90 mM in the $Ca^{2+}$-deficient medium (Table 1). This doubled the intracellular content of $K^+$ and decreased the $Na^+$ content to about one-third, but had little effect on leucine incorporation. However, when the external $Mg^{2+}$ concentration in this medium was increased 4-fold with a resultant increase in cellular $Mg^{2+}$ content of about 20%, the leucine incorporation rate increased 5-fold and exceeded the rate found in the normal, $Ca^{2+}$-sufficient medium.

An experiment was done to determine the relationship between intracellular $Mg^{2+}$ and protein synthesis in cells incubated with graded concentrations of $Mg^{2+}$. The results (Fig. 6) show that small increases of intracellular $Mg^{2+}$ up to about 0.08 μmol per mg of protein produced large increases in the rate of protein synthesis. When the intracellular $Mg^{2+}$ exceeded 0.12 μmol per mg of protein, protein synthesis was inhibited.

Table 1. Effects of altering intracellular $K^+$ and $Na^+$ on rate of protein synthesis

| Group | Cations in medium, mM | | | | Cations in cells, μmol/mg protein | | | | [$^3$H]Leucine, cpm/μg protein |
|---|---|---|---|---|---|---|---|---|---|
| | $Ca^{2+}$ | $K^+$ | $Na^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $K^+$ | $Na^+$ | $Mg^{2+}$ | |
| A | 1.53 | 4.74 | 149 | 0.99 | 0.011 | 0.888 | 0.134 | 0.067 | 12.16 |
| B 1 | 0.014 | 4.84 | 152 | 0.97 | 0.008 | 0.286 | 0.474 | 0.060 | 2.87 |
| 2 | * | 18.07 | 130 | * | * | 0.339 | 0.425 | 0.054 | 3.82 |
| 3 | * | 45.31 | 104 | * | * | 0.480 | 0.336 | 0.053 | 4.09 |
| 4 | * | 71.35 | 75 | * | * | 0.563 | 0.243 | 0.053 | 3.96 |
| 5 | * | 90.38 | 60 | * | * | 0.555 | 0.167 | 0.050 | 4.06 |
| C | * | 4.80 | 144 | 4.24 | * | 0.877 | 0.079 | 0.072 | 14.29 |

Confluent cultures were washed and incubated in medium with 10% dialyzed calf serum containing the indicated cation concentrations. Group A was a physiological control; group B was deficient in $Ca^{2+}$ and contained increasing concentrations of $K^+$ with compensating decreases in $Na^+$; group C was deficient in $Ca^{2+}$ and contained physiological $K^+$ and $Na^+$ and a supranormal concentration of $Mg^{2+}$. At 3 hr the cultures were processed for measurement of cations or [$^3$H]leucine incorporation.
*No significant variation in measured concentration of $Ca^{2+}$ or $Mg^{2+}$.

## DISCUSSION

A composite view of the results shows a direct and possibly causal relationship between the concentration of $Mg^{2+}$ (in $Ca^{2+}$-deficient medium), the rate of protein synthesis, and the onset of DNA synthesis. The rate of protein synthesis 3 hr after medium change increases sharply with extracellular $Mg^{2+}$ up to 5 mM, increases more slowly up to 20 mM, and decreases at higher concentrations (Fig. 1). The rate of DNA synthesis at 17 hr increases sharply up to 20 mM $Mg^{2+}$ and decreases at higher concentrations. There is a seeming discrepancy between the broad range of $Mg^{2+}$ concentrations (5–20 mM) that maximizes protein synthesis at 3 hr and the narrower and higher (≈20 mM) range that maximizes DNA synthesis as measured at 17 hr. This discrepancy can be explained by the fact that the $Mg^{2+}$ concentrations in the medium that are required to maximize protein synthesis increase with time (Figs. 3 and 5). This is apparently because the cells tend to restore their intracellular [$Mg^{2+}$] toward normal levels after 3 hr (Fig. 5).

The effects of extracellular [$Mg^{2+}$] on protein synthesis are strikingly evident within 30–45 min after the shift to $Ca^{2+}$-deficient medium (Fig. 2). Because the intracellular content of $Mg^{2+}$ itself changes gradually in response to external $Mg^{2+}$ concentration for the first 3 hr (12), it is plausible that the change in the rate of protein synthesis is a direct response to the intracellular $Mg^{2+}$ content. The rate of DNA synthesis, however, does not change until more than 10 hr after the medium change (Fig. 4*b*), and, therefore, it is likely to be an indirect effect of the intracellular cationic changes. When there is a delay in the stimulation of protein synthesis as is the case with 35 mM [$Mg^{2+}$] in Fig. 4*a*, there is a similar delay in the onset of DNA synthesis (Fig. 4*b*). These observations, taken together, indicate that the altered cellular cations primarily influence the rate of protein synthesis, which later plays a significant role in determining the onset of DNA synthesis.

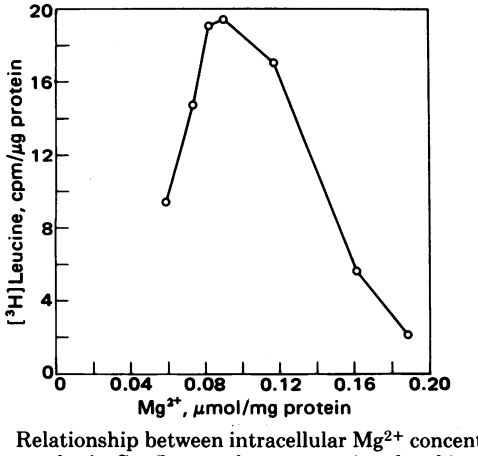


FIG. 6. Relationship between intracellular $Mg^{2+}$ concentration and protein synthesis. Confluent cultures were rinsed and incubated in media containing varying concentrations of $Mg^{2+}$ in 0.02 mM $Ca^{2+}$ for 3 hr. Some of the cultures were then labeled for 1 hr with 1 μCi of [$^3$H]leucine per ml, or were washed with a solution containing 150 mM NaCl and 1.0 mM $CaCl_2$ and processed for atomic absorption spectrophotometry to determine intracellular $Mg^{2+}$ content.

It is important to sort out which of the four major cations is actually responsible for the altered rates of protein synthesis. The simple omission of $Ca^{2+}$ from culture medium is accompanied by a marked drop in the rate of protein synthesis in the cells, but there is no significant decrease of intracellular $Ca^{2+}$ (Fig. 5). The rate of protein synthesis in $Ca^{2+}$-deficient medium is restored to maximal levels by raising the concentration of $Mg^{2+}$, which raises the intracellular $Mg^{2+}$ content but slightly lowers the intracellular $Ca^{2+}$ content (Fig. 5). This is consistent with the view that $Mg^{2+}$ is the direct effector of protein synthesis, and $Ca^{2+}$ only influences the availability of $Mg^{2+}$. A similar conclusion was drawn in studying the effects of divalent cations on the uptake of uridine by 3T3 cells. The uptake of uridine is limited by the phosphorylation of uridine and is part of the coordinate response of 3T3 cells to serum (12). Uridine uptake is sharply inhibited by omission of $Ca^{2+}$ and $Mg^{2+}$ together, and can be restored to maximal rates by replacing $Mg^{2+}$, but is unaffected by replacing $Ca^{2+}$. One of the major effects of omitting $Ca^{2+}$ is to increase the exchangeability of $Mg^{2+}$ between cells and medium (12). In modulating the diverse reactions of the coordinate response of chicken embryo cells, $Sr^{2+}$ can substitute for $Ca^{2+}$, but no substitute for $Mg^{2+}$ has been found (18). We have interpreted this together with other results to indicate that $Ca^{2+}$ is acting at the level of the cell membrane, whereas $Mg^{2+}$ acts directly on the diverse reactions of the coordinate response. Another indication that $Mg^{2+}$ acts more proximally than $Ca^{2+}$ on the coordinate response is the observation that cellular DNA synthesis that has been inhibited by severe deprivation of $Ca^{2+}$ in the presence of physiological [$Mg^{2+}$] can be stimulated by supranormal concentrations of $Mg^{2+}$, whereas the reverse is clearly not true (13). Furthermore, $Ca^{2+}$ is not known to play a direct role in any of the biochemical reactions of the coordinate response, whereas $Mg^{2+}$ plays a role in many if not all of them, and plays a particularly important role in protein synthesis at every level from charging tRNA (19) through initiating protein synthesis (20) and maintaining the conformation of ribosomes (21). These findings lend further weight to the evidence against a direct role of $Ca^{2+}$ as the effector of the coordinate response, but are compatible with such a role for $Mg^{2+}$.

Unlike $Ca^{2+}$, $K^+$ is required for protein synthesis (20), al-

though it is not required for some other reactions of the coordinate response (12). Maximum protein synthesis in cell-free systems has a much broader peak for $K^+$ than for $Mg^{2+}$, and $NH_4^+$ can substitute for $K^+$ in this function (20).

A drastic decrease in intracellular $K^+$ does inhibit protein synthesis (22), but this decrease in $K^+$ is far greater than any that occurs in response to physiological effectors (refs. 14 and 15; Fig. 5). The decrease in cellular $K^+$ content that occurs at 3 hr in $Ca^{2+}$-deficient medium cannot be responsible for the accompanying inhibition of protein synthesis, because raising the cellular $K^+$ level by adding high concentrations to the medium is without effect (Table 1). Also, as noted above, the cell restores its normal $K^+$ content by 17 hr without raising the rate of protein synthesis (Fig. 5). There is no change in cellular $K^+$ at the ultrahigh concentrations of $Mg^{2+}$ that inhibit protein synthesis. Therefore, a primary role for $K^+$ in regulating protein synthesis under the conditions used here appears most unlikely. However, we do find that physiological stimuli that raise intracellular $Mg^{2+}$ levels also raise $K^+$ levels (12, 14, 15, 17), and it remains a possibility that $K^+$ augments the effects of $Mg^{2+}$ (20). There is no evidence that $Na^+$ is required for or affects protein synthesis in cell-free systems, and our results fail to show any correlation between cellular $Na^+$ content and protein synthesis.

The critical question remains whether the availability of $Mg^{2+}$ changes sufficiently with physiological effectors to account for their effects on protein synthesis and other elements of the coordinate response. We have found that the $Mg^{2+}$ content of chicken embryo cells stimulated by insulin and 3T3 cells stimulated by serum increases by about 15% (14, 15). A similar increase occurs in uterine tissue treated with insulin (23). Increased availability of $Mg^{2+}$ is also thought to occur in skeletal muscle stimulated by insulin (24) and in heart muscle in a work jump (25). In addition to an increase in the overall cellular content of $Mg^{2+}$, a redistribution of $Mg^{2+}$ already within the cell probably occurs (25). Any change in the availability of $Mg^{2+}$ within the cell is likely to influence the rate of protein synthesis because the estimated free $Mg^{2+}$ of the cell (26) is lower than the optimal concentration for initiation of protein synthesis (20). The change in availability of $Mg^{2+}$ required to significantly alter the rate of protein synthesis is a small one. In the experiment of Fig. 6, an increase in cellular $Mg^{2+}$ content of only 12% (from 0.073 to 0.082 $\mu$mol/mg of protein) increases the rate of protein synthesis by 33%. Both values are within the physiological range of cellular $Mg^{2+}$ content, and the increase is similar in magnitude to that produced by serum in these cells (13). The shape of the curve for protein synthesis as a function of $Mg^{2+}$ in the cells (Fig. 6) is strikingly similar to that seen in cell-free protein synthesis (20), because both include a steep upward limb at low $Mg^{2+}$ and a fairly sharp peak and a more gradual downward limb at higher $Mg^{2+}$ concentrations. It appears that any small change in availability of $Mg^{2+}$ in the cell will influence the rate of protein synthesis, and, therefore, the proposal that $Mg^{2+}$ plays a role in physiological regulation of protein synthesis is a reasonable one.

The results indicate that protein synthesis could play an important role in determining the onset of DNA synthesis. In some cellular systems, however, the rate of protein synthesis is not changed by serum treatment (27). Because the cells in these systems multiply in response to the serum, it is apparent that serum increases the rate of protein accumulation; in the absence of increased protein synthesis there must therefore be a decrease in the rate of protein degradation. In other systems, including 3T3 cells (9, 28), both synthesis and degradation of proteins in serum-stimulated cells are so affected as to maximize the accumulation of protein, and in a third category there are cells in which only protein synthesis is affected by serum (29). Because protein accumulation must parallel DNA accumulation for balanced growth, both protein synthesis and degradation may be involved in regulating cell multiplication. Although $Mg^{2+}$ may be involved in regulating protein synthesis, we do not find evidence that it affects the rate of degradation (data not shown). This suggests that the serum inhibition of degradation is brought about by some mechanism quite different from its stimulation of protein synthesis.

McKeehan and Ham (30) concur in our conclusion that $Mg^{2+}$ is more directly involved than $Ca^{2+}$ in regulating the intracellular events that lead to cell replication. They also conclude that the "transformation of cells causes a selective loss of the regulatory role of $Mg^{2+}$ but not $Ca^{2+}$ in cellular multiplication."

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1. Rubin, H. & Koide, T. (1975) *J. Cell Physiol.* **86,** 47–58.
2. Rozengurt, E., Stein, W. & Wigglesworth, N. (1977) *Nature (London)* **267,** 442–444.
3. Fodge, D. & Rubin, H. (1973) *Nature (London) New Biol.* **246,** 181–183.
4. Moscatelli, D. & Rubin, H. (1977) *J. Cell. Physiol.* **91,** 79–88.
5. Kamine, J. & Rubin, H. (1977) *J. Cell. Physiol.* **92,** 1–12.
6. Rubin, H. & Fodge, D. (1974) in *Control of Proliferation in Animal Cells,* eds. Clarkson, B. & Baserga, R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 810–816.
7. Rubin, H. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3551–3555.
8. Rubin, H. (1976) *J. Cell. Physiol.* **89,** 613–625.
9. Hershko, A., Mamont, P., Shields, R. & Tomkins, G. M. (1971) *Nature (London) New Biol.* **232,** 206–211.
10. Tomkins, G. & Gelehrter, T. (1972) in *Biochemical Actions of Hormones,* ed. Litwack, G. (Academic, New York), pp. 1–20.
11. Rubin, H. & Koide, T. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 168–172.
12. Bowen-Pope, D. F., Vidair, C., Sanui, H. & Rubin, A. H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1308–1312.
13. Rubin, A. H., Terasaki, M. & Sanui, H. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4379–4383.
14. Sanui, H. & Rubin, H. (1978) *J. Cell. Physiol.* **96,** 265–278.
15. Sanui, H. & Rubin, A. H. (1978) *J. Cell. Biol.* **79,** 84a.
16. Vogt, M. & Dulbecco, R. (1963) *Proc. Natl. Acad. Sci. USA* **49,** 171–179.
17. Sanui, H. & Rubin, H. (1977) *J. Cell. Physiol.* **92,** 23–32.
18. Rubin, H. (1977) *J. Cell. Physiol.* **91,** 449–458.
19. Novelli, G. D. (1967) *Annu. Rev. Biochem.* **36,** 449–484.
20. Schreier, M. H. & Staehlin, T. (1973) *J. Mol. Biol.* **73,** 329–349.
21. Meselson, M., Nomura, M., Brenner, S., Davern, C. & Schlessinger, D. (1964) *J. Mol. Biol.* **9,** 696–711.
22. Ledbetter, M. & Lubin, M. (1977) *Exp. Cell. Res.* **105,** 223–236.
23. Lostroh, A. J. & Krahl, M. E. (1978) *Adv. in Enzyme Regulation* **12,** 73–81.
24. Peck, E. & Ray, W. (1971) *J. Biol. Chem.* **246,** 1160–1167.
25. Kohn, M. C., Achs, M. J. & Garfinkel, D. (1977) *Am. J. Physiol.* **232,** R158–R163.
26. Gupta, R. K., Benovic, J. L. & Rose, Z. B. (1978) *J. Biol. Chem.* **253,** 6172–6176.
27. Baenziger, N. L., Jacobi, C. H. & Thach, R. E. (1974) *J. Biol. Chem.* **249,** 3483–3488.
28. Castor, L. N. (1977) *J. Cell Physiol.* **92,** 457–468.
29. Baxter, G. C. & Stanners, C. P. (1978) *J. Cell. Physiol.* **96,** 139–146.
30. McKeehan, W. L. & Ham, R. G. (1978) *Nature (London)* **275,** 756–758.