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Separate roles for calcium and magnesium in their synergistic effect on uridine uptake by cultured cells: Significance for growth control

(membrane permeability/transport and phosphorylation/DNA synthesis/intracellular Na^+, K^+)

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ABSTRACT **The uptake of uridine by BALB/c3T3 cells is markedly inhibited by reducing the concentration of Mg^{2+} in medium containing only traces of Ca^{2+}. When physiological [Ca^{2+}] is present in the medium, omission of Mg^{2+} has no effect on uridine uptake, and when Mg^{2+} is present, omission of Ca^{2+} has only a slight inhibitory effect. When both Ca^{2+} and Mg^{2+} are omitted, the concentration of Ca^{2+} in the cells is not reduced, but that of Mg^{2+} is reduced to about one-half in 3 hr. The concentration of K^+ is also reduced, and that of Na^+ is increased, suggesting increased membrane permeability to cations. The rate of diffusion of the nontransported hexose, L-glucose, into the cells is greatly increased. Changes in intracellular Na^+ and K^+ concentrations do not in themselves affect uridine uptake. When Ca^{2+} alone is restored to the medium of cells that had been deprived of both Ca^{2+} and Mg^{2+}, there is no increase in the greatly depressed rate of uridine uptake, but when Mg^{2+} alone is restored, the rate of uridine uptake returns to control values. We conclude that the omission of Ca^{2+} from the medium has no direct effect on uridine uptake, but acts by increasing the exchange of Mg^{2+} between cells and medium and by otherwise altering the availability of Mg^{2+} for this reaction. A similar conclusion is reached in considering the role of these ions in the regulation of other reactions of the coordinate response, including the initiation of DNA synthesis and the control of growth.**

The omission of either Ca^{2+} or Mg^{2+} from the medium of cultured cells inhibits metabolism and growth to varying degrees in different cell types (1–7). The effects of omitting one can be greatly intensified by reducing the concentration of the other (4, 7, 8), and it is not completely clear whether the two are interchangeable in some cellular function(s) or have separate roles. Our studies of the divalent cations have concentrated on their effects on the initiation of DNA synthesis, which is a delayed response of cells to external effectors and dependent on other cellular activities, including energy metabolism and the synthesis of RNA and protein (9). Because the cellular activity that controls the onset of DNA synthesis is unknown, it is difficult to determine the respective roles of the two cations. The evidence accumulated to date indicates, but does not prove, that Mg^{2+} is the more proximal of the two cations in regulating the onset of DNA synthesis and that Ca^{2+} influences the availability of Mg^{2+} (4, 6, 8). We have done more limited studies of the Ca^{2+} and Mg^{2+} requirements of simpler reactions of the coordinate response of cells to external effectors (5, 8, 10). These reactions include the transport of hexoses and the uptake of uridine, which accelerate within minutes after addition of the same external effectors that later stimulate the onset of DNA synthesis. The results suggested that Mg^{2+} is the more proximal effector of the early responses and that Ca^{2+} exerts its effect through Mg^{2+}.

It has recently been shown that the uptake of uridine in several lines of cultured cells is limited by the rate of its phosphorylation rather than its transport (11–13). Since the *in vitro* rate of uridine phosphorylation varies with [Mg^{2+}] and is unaffected by Ca^{2+} (ref. 14; unpublished observation), a direct role for Mg^{2+} in regulating uridine uptake became highly plausible and a similar role for Ca^{2+} unlikely. We decided to examine further the respective roles of the two ions in the regulation of uridine uptake because such a study promised to provide a clear-cut distinction between them. The basic strategy was to produce a powerful inhibition of uridine uptake by omitting both Ca^{2+} and Mg^{2+} from the medium and to observe the effect of restoring them singly or together. The effects of varying Na^+ and K^+ were also studied. The studies of uridine uptake were accompanied by measurements of the four major cations within the cells and of the cellular permeability to L-glucose. The results provide strong evidence for the direct role of Mg^{2+} in regulating uridine uptake and indicate the importance of Ca^{2+} in maintaining the permeability barrier of the cell. No evidence was found for a regulatory role of either Na^+ or K^+ in uridine uptake.

MATERIALS AND METHODS

Cell Culture and Labeling. BALB/c3T3 cells were maintained in modified Eagle's medium with 10% calf serum and labeled with [^{3}H]thymidine as described (8). 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 overnight before use in experiments. In most experiments, cultures were labeled by addition of [^{3}H]uridine (13.3 Ci/mmol, 1 Ci = 3.70×10^{10} Bq) for varying periods in the appropriate experimental medium and washed three times with ice-cold Tris-buffered saline. Acid-soluble material was extracted for 15 min with cold 5% trichloroacetic acid for scintillation counting. In the experiment of Fig. 1, however, cultures were washed five times by dipping in five successive beakers containing ice-cold 150 mM NaCl before extraction in trichloroacetic acid. The cultures were then dissolved in 0.1 M NaOH for subsequent measurement of protein content by the method of Lowry *et al.* (15) and, in the experiment of Fig. 6, of acid-insoluble radioactivity by scintillation counting.

The uptake of L-glucose was measured in the various experimental media by addition of 5 μCi of L-[^{3}H]glucose at tracer concentrations (10). After 20–40 min, the cultures were washed five times with Tris-buffered saline and material was extracted in cold 5% trichloroacetic acid for scintillation counting. Correction was made for extracellular label by subtracting the trichloroacetic acid-soluble counts remaining with the cells in physiological medium after 10 sec of incubation followed by washing. A similar correction was made for extracellular label in the uridine uptake experiment of Fig. 1.

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Modified Eagle's medium was prepared for experimental manipulations without added Ca^{2+} or Mg^{2+}. Serum was dialyzed against physiological saline free of Ca^{2+} and Mg^{2+}. The medium with 10% serum contained 0.015–0.020 mM of contaminating Ca^{2+} and Mg^{2+}, as determined by atomic absorption spectrophotometry.

The procedures for measuring cation contents of cells by atomic absorption spectrophotometry were as reported (16). Briefly, cultures were washed five times with 10 ml per wash of CO_2-free 0.25 M sucrose solution, approximately pH 7. They were then exposed to a carbonated sucrose solution (pH 4) to displace the externally bound cations. The cells were then scraped from the dish and diluted 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

Effects of Ca^{2+}, Mg^{2+}, and Serum on Uridine Uptake. In the absence of serum, the rate of uptake of uridine by 3T3 cells is limited largely by its phosphorylation (11, 17). This limit is expressed by a marked decrease with time in the slope of the curve for uridine uptake (Fig. 1 and refs. 11 and 17). A similar decrease in slope is produced by drastically lowering the concentrations of Ca^{2+} and Mg^{2+} in medium containing 10% serum. Addition of supranormal $[Mg^{2+}]$ to the low $[Ca^{2+}]$ serum-containing medium restores uridine uptake to the high levels obtained in the presence of physiological concentrations of Ca^{2+} and Mg^{2+} in 10% serum. The results indicate that the marked reductions of Ca^{2+} and Mg^{2+} concentrations inhibit uridine phosphorylation, as does omission of serum. Restoration of normal rates of uptake in the low $[Ca^{2+}]$ medium by high $[Mg^{2+}]$ is consistent with the view (10) that Mg^{2+} is the major effector of this process.

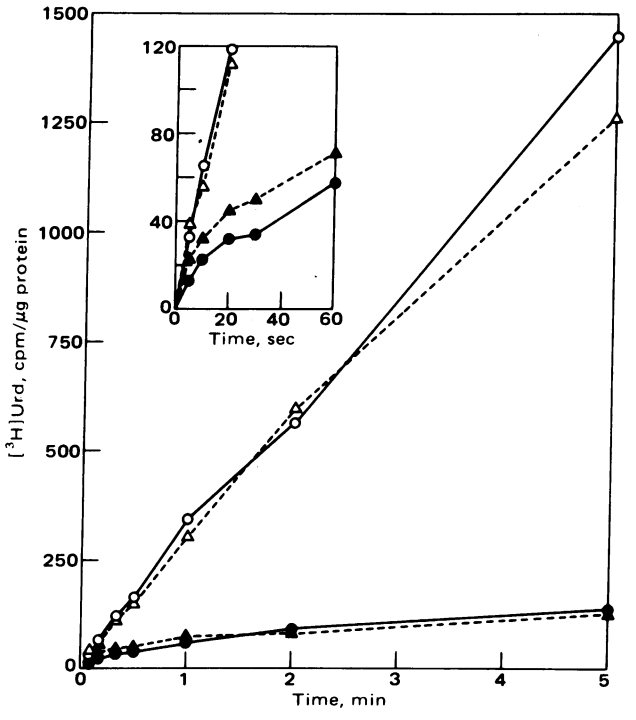


FIG. 1. Kinetics of uridine uptake with variations in divalent cations or serum. BALB/c3T3 cells were grown to confluency in 35-mm dishes and switched to 1% serum overnight. They were washed twice with Tris/saline buffer and incubated in the various experimental media for 3 hr. The experimental media were buffered at pH 7.4 with 25 mM Hepes, and the cultures were partly immersed in a 37°C waterbath throughout the experiment. At 3 hr, fresh experimental media containing 23 μCi of [³H]uridine per ml were added for the appropriate intervals. The cultures were then washed, the acid-soluble material was extracted for scintillation counting, and the protein content was determined. The amount of [³H]uridine that could be accounted for by residual extracellular labeling medium was calculated by using L-[³H]glucose and was subtracted from the experimental data. In 10% serum: O—O, 1.7 mM Ca^{2+}, 0.8 mM Mg^{2+}; Δ---Δ, 0.02 mM Ca^{2+}, 10.0 mM Mg^{2+}; ▲---▲, 0.02 mM Ca^{2+}, 0.1 mM Mg^{2+}. In 0% serum: ●—●, 1.7 mM Ca^{2+}, 0.8 mM Mg^{2+}.

The rate of uridine accumulation for a period of 10 min or more has been shown to depend on the rate of phosphorylation of uridine (17). In the experiments to follow, we adopted a labeling period of 10–15 min as a measure of the rate of phosphorylation.

Dependence of Uridine Uptake on $[Mg^{2+}]$ and [Serum]. The requirements for serum and for Mg^{2+} in uridine uptake are considerably less than their requirements in the initiation of DNA synthesis (Fig. 2). Furthermore, ultrahigh concentrations of Mg^{2+} (40 mM) inhibit the onset of DNA synthesis, but do not inhibit uridine uptake. These findings show that different Mg^{2+}-dependent processes are involved in controlling the uptake of uridine and the onset of DNA synthesis.

Effects of Extracellular Ca^{2+} and Mg^{2+} on Intracellular Cation Content. Removal of Mg^{2+} from the medium has little effect on either intracellular cation concentrations or uridine uptake relative to the controls (Fig. 3). Removal of Ca^{2+} alone causes a marked decrease in $[K_i^+]$ and a similar increase in $[Na_i^+]$, indicating a breach in membrane integrity. There is a slight decrease in $[Mg_i^{2+}]$ at 3 hr and, paradoxically, a slight increase in $[Ca_i^{2+}]$ despite its omission from the medium. The residual $[Ca^{2+}]$ in medium to which none has been added is higher than the estimated concentration of free Ca^{2+} within the cells. Thus the inward Ca^{2+} activity gradient is maintained even in low Ca^{2+} medium, while the permeability of the cells is increased, a situation that might allow cellular Ca^{2+} to increase. When both Mg^{2+} and Ca^{2+} are omitted from the medium, there is a complete reversal of K^+ and Na^+ concentrations in the cell. $[Mg_i^{2+}]$ decreases by about one-half, showing that the omission of both Ca^{2+} and Mg^{2+} from the medium makes the cell much more permeable to Mg^{2+} than does the omission of Mg^{2+} alone. Despite the omission of both Mg^{2+} and Ca^{+}, however, there is no loss of intracellular Ca^{2+}. When Ca^{2+} is omitted but $[Mg_o^{2+}]$ is raised to 13 mM, the loss of K^+ and increase of Na^+ are prevented, $[Mg_i^{2+}]$ is increased, and $[Ca_i^{2+}]$ is slightly decreased.

Uridine uptake was increased at 1 hr after medium change despite the omission of either one or both of the divalent cations.

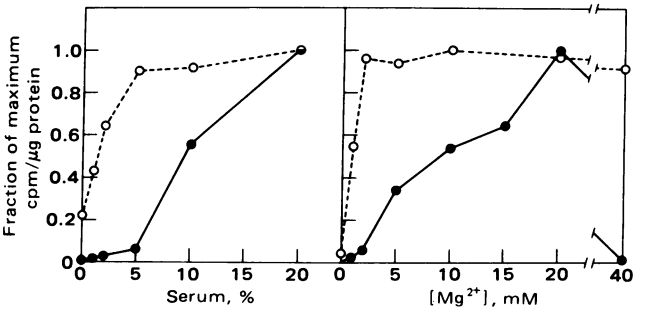
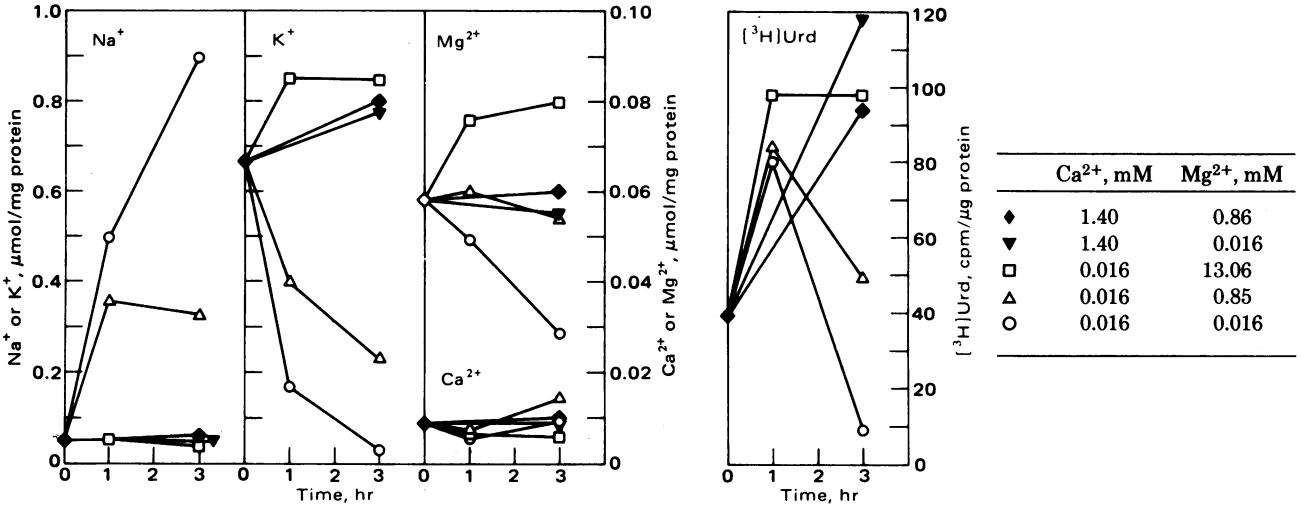


FIG. 2. Serum and Mg^{2+} dependency of uridine uptake and onset of DNA synthesis. Six-day-old cultures, which had been in 1% calf serum overnight, were switched to modified Eagle's medium with 10% dialyzed calf serum plus the indicated concentrations of Mg^{2+} and 0.02 mM Ca^{2+}. After 3 or 17 hr of incubation, the cultures were labeled with [³H]uridine or [³H]thymidine, respectively. O---O, Acid-soluble [³H]uridine; ●—●, acid-insoluble [³H]thymidine.



	Ca^{2+}, mM	Mg^{2+}, mM
♦	1.40	0.86
▼	1.40	0.016
□	0.016	13.06
△	0.016	0.85
○	0.016	0.016

FIG. 3. Change in intracellular cation content and uridine uptake with variations of extracellular Ca^{2+} and Mg^{2+}. Confluent cultures in 100-mm dishes were incubated in the appropriate media for the indicated times. Intracellular cation content and the rate of [^{3}H]uridine uptake into acid-soluble material were determined.

This reflects the response of the cells to the increased concentration of serum in the fresh medium, since there is no increase when serum is omitted (8). It appears that the early response of uridine uptake to serum stimulation manifests itself before the inhibitory effects of external ion deprivation are felt in the cell. The latter manifest themselves at 3 hr, when there has been significant loss of Mg^{2+} from the cells.

When $[Mg_o^{2+}]$ is increased in a graded fashion in low$[Ca_o^{2+}]$, there is a graded increase in $[K_i^+]$, $[Mg_i^{2+}]$, and uridine uptake, with a decrease in $[Na_i^+]$ and little change in $[Ca_i^{2+}]$ (Fig. 4). In the low Ca^{2+} medium an increase of more than 5-fold in $[Mg_o^{2+}]$ over physiological concentration is required to restore $[K_i^+]$ and $[Na_i^+]$ to control levels. The omission of Ca^{2+} in the presence

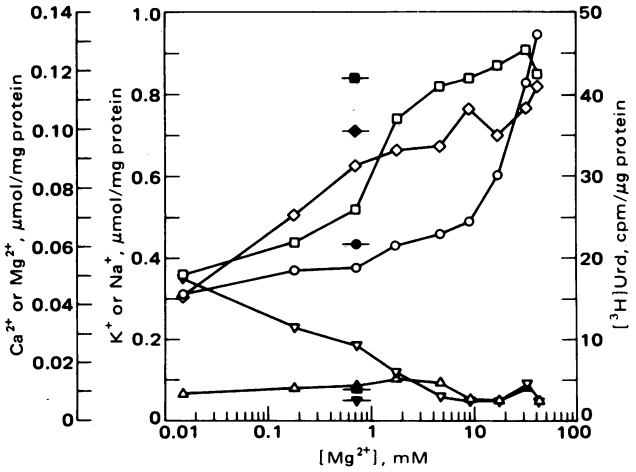


FIG. 4. Change in intracellular cation content with graded concentrations of extracellular Mg^{2+}. Confluent cultures in 100-mm dishes were incubated for 3 hr in modified Eagle's medium containing 10% dialyzed calf serum with no added Ca^{2+} and varying concentrations of Mg^{2+} (abscissa) or with physiological concentrations of both. Intracellular cation content and the rate of uptake for 15 min of [^{3}H]uridine into acid-soluble material were determined. Open symbols, no added Ca^{2+} (Ca^{2+} = 0.017–0.020 mM); closed symbols, control Ca^{2+} (Ca^{2+} = 1.46 mM). Intracellular cations: ▽ and ▼, Na^+; □ and ■, K^+; △ and ▲, Ca^{2+}; ○ and ●, Mg^{2+}. ◇ and ♦, uptake of [^{3}H]uridine.

of physiological $[Mg_o^{2+}]$ results in a slight decrease of $[Mg_i^{2+}]$, but not in $[Ca_i^{2+}]$.

Effects of Na^+ and K^+ on Uridine Uptake. As external $[Mg^{2+}]$ is increased in Figs. 3 and 4, cellular K^+ is increased and cellular Na^+ decreased. This raised the possibility that uridine uptake was responding to the monovalent rather than the divalent cations. The effects of Na^+ and K^+ were therefore tested directly by varying their concentrations in the medium and measuring cellular cations and uridine uptake. Reduction of K^+ in the medium to 0.22 mM caused a decrease in cellular K^+ to $^1/_{10}$th its original value and a 10-fold increase in cellular Na^+ without affecting the uptake of uridine (Table 1). An increase of external K^+ to 134.7 mM with a corresponding decrease in external Na^+ caused a significant increase in cellular K^+, but *decreased* uridine uptake. When external Na^+ was reduced and substituted with choline instead of K^+, the decreased uptake of uridine was largely eliminated. These results show that the changes in cellular Na^+ and K^+ that occur when the divalent cations are varied cannot account for the altered rates of uridine uptake.

Cell Permeability Changes with Variations in $[Ca_o^{2+}]$ and $[Mg_o^{2+}]$. L-Glucose enters the cell only by nonmediated diffusion. It is therefore useful as an indicator of changes in the permeability of the cell to small uncharged molecules. The effects of variations in $[Ca_o^{2+}]$ and $[Mg_o^{2+}]$ on the uptake of L-glucose are shown in Fig. 5. In physiological concentrations of Ca^{2+}, the diffusion of L-glucose into the cell is slow and is unaffected by changes in $[Mg_o^{2+}]$. By contrast, omission of Ca^{2+} from the medium causes a marked increase in the uptake of L-glucose even in a physiological concentration of Mg^{2+}, and this is greatly increased by lowering $[Mg_o^{2+}]$. The uptake in low Ca^{2+} can be reduced to low levels, however, by increasing $[Mg_o^{2+}]$ to 15 mM.

Restoration of Ca^{2+} or Mg^{2+} or Both to Cells Deprived of Both. From the fact that omission of Ca^{2+} inhibits uridine uptake while the omission of Mg^{2+} does not, one might mistakenly assume that Ca^{2+} is the more important of the two for this function. However, the omission of both cations causes a much larger inhibition of uridine uptake than the omission of Ca^{2+} alone, which in any case, does not reduce $[Ca_i^{2+}]$ (Figs. 3 and 4).

Addition of Ca^{2+} to cultures that had been deprived of both Ca^{2+} and Mg^{2+} for 1 hr greatly retarded further decline in the

Table 1. Effects of varying extracellular Na^+ and K^+ on intracellular cations and uptake of uridine

Cation	Medium* A	B	C	D	E†
			Medium,* mM		
Na^+	133.4	134.1	73.9	6.75	7.25
K^+	0.22	2.75	69.9	134.7	4.91
			Cells, μmol/mg of protein		
Na^+	0.853	0.060	0.045	0.040	0.030
K^+	0.060	0.881	0.878	1.216	0.650
Ca^{2+}	0.028	0.021	0.022	0.023	0.022
Mg^{2+}	0.061	0.059	0.056	0.050	0.056
			Uridine uptake, cpm/μg of protein		
	150.0	136.4	128.6	73.0	114.9

Cultures in 100-mm dishes were incubated in medium containing 10% dialyzed calf serum and varying concentrations of Na^+ and K^+ or choline. At 3 hr the cation content of the cells and the 15-min uptake of [^{3}H]uridine into acid-soluble material were determined. The measured cation content of each of the five media A–E and of the cells in those media and the uptake of uridine are shown.

* All media contained 1.40 mM Ca^{2+} and 0.83 mM Mg^{2+}.

† Medium E contained 140 mM choline.

rate of uridine uptake into the trichloroacetic acid-soluble pool (Fig. 6). Addition of Ca^{2+} to the doubly deprived cultures at 2 hr, however, failed to stimulate the greatly depressed rate of uridine uptake. Addition of 5 mM Mg^{2+} at either 1 or 2 hr restored the uptake of uridine into the trichloroacetic acid-soluble pool almost to control levels. Addition of physiological [Mg^{2+}] alone was more effective than addition of physiological concentrations of both Ca^{2+} and Mg^{2+}. The same trends are seen in the incorporation of uridine into acid-insoluble material except that the restoration by Mg^{2+} is not as complete as in the acid-soluble pools. The capacity of Ca^{2+} to retard further inhibition of uridine uptake at 1 hr is probably related to the prevention of the further loss of Mg^{2+} which would otherwise occur in low Ca^{2+} medium (see Fig. 3). At 3 hr, however, Mg^{2+} has been reduced by 30–60% (Figs. 3 and 4) and Ca^{2+} has no restorative power on uridine uptake, presumably because Mg^{2+} is the critical component. The fact that Mg^{2+} alone is more effective at restoring uridine uptake than are Ca^{2+} and Mg^{2+} together may be due to resealing of the membrane by Ca^{2+}, thus retarding entry of Mg^{2+}.

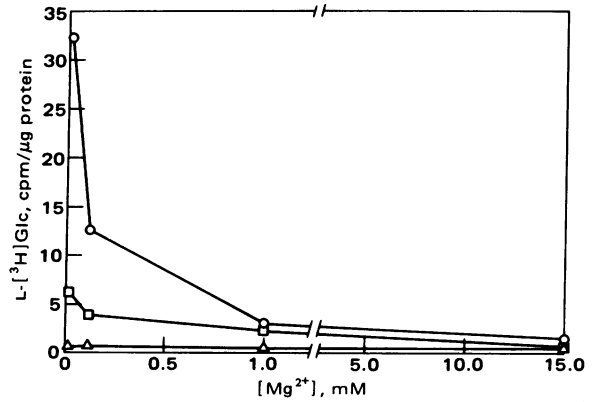


FIG. 5. Uptake of L-glucose by cells in various concentrations of Ca^{2+} and Mg^{2+}. Confluent cultures were incubated for 3 hr in modified Eagle's medium containing 10% dialyzed calf serum with varying amounts of Ca^{2+} and Mg^{2+}, and the rates of uptake of L-[^{3}H]glucose for 20 min were measured in the same media. [Ca^{2+}]: ○, 0.02 mM; □, 0.05 mM; △, 1.7 mM.

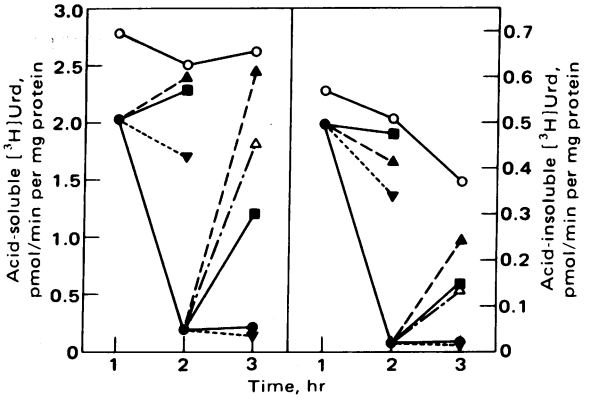


FIG. 6. Uridine uptake and incorporation after restoration of Ca^{2+} or Mg^{2+} or both to cultures deprived of both. Confluent cultures were incubated in medium with no added Ca^{2+} or Mg^{2+} for 1–3 hr. At 1 and 2 hr, varying amounts of Ca^{2+} or Mg^{2+} or both were restored to the media as indicated below and the cultures were incubated for an additional hour. They were then labeled for 10 min with [^{3}H]uridine, and intracellular acid-soluble (*Left*) and acid-insoluble (*Right*) [^{3}H]uridine was determined. Control cultures containing the physiological concentrations of Ca^{2+} and Mg^{2+} throughout the experiment were labeled at hourly intervals. ○—○, 1.7 mM Ca^{2+}, 1.0 mM Mg^{2+} throughout; ●—●, 0.02 mM Ca^{2+}, 0.02 mM Mg^{2+} throughout. Cultures in 0.02 mM Ca^{2+} and 0.02 mM Mg^{2+} for 1 or 2 hr and the following concentrations of Ca^{2+} or Mg^{2+} or both added for an additional hr: ■—■, 1.7 mM Ca^{2+}, 1.0 mM Mg^{2+}; ▼---▼, 5.0 mM Ca^{2+}; △- - -△, 1.0 mM Mg^{2+}; ▲- - -▲, 5.0 mM Mg^{2+}.

DISCUSSION

The omission of Ca^{2+} from the medium produces a striking increase in cell permeability, which is accentuated by lowering [Mg_o^{2+}]. This is made apparent by an increase in [Na_i^+], a decrease in [K_i^+], and an increase in uptake of L-glucose. It is also manifest in the finding that Mg^{2+} is much more easily removed from the cell by lowering [Mg_o^{2+}] in the absence, than in the presence, of Ca^{2+}. Indeed, in physiological [Ca_o^{2+}] there is only about a 10% loss of Mg^{2+} from the cell when Mg^{2+} is omitted from the medium and there is no significant change in [Na_i^+] and [K_i^+]. Paradoxically, Ca^{2+} is the only major intracellular cation that is not consistently changed by omitting Ca^{2+} from the medium. This may be related to the fact that estimates of free [Ca_i^{2+}] ($<10^{-7}$ M) are lower than the residual [Ca_o^{2+}] of about 0.02 mM in medium to which it has not been deliberately added. It points up the danger of assuming that changes occur in the concentration of a particular ion within a cell just because its extracellular concentration is changed. However, omission of Ca^{2+} from the medium does cause a decrease in Ca^{2+} bound to the external surface of the cell to 25% of its original value (8). The loss of surface-bound Ca^{2+} probably contributes to the increased permeability of the cell membrane. Mg^{2+} apparently can substitute for Ca^{2+} in maintaining normal permeability, but it does so inefficiently, since 5–10 mM Mg_o^{2+} is required to keep Na_i^+ and K_i^+ at normal levels in the absence of Ca^{2+}.

The different roles of Ca^{2+} and Mg^{2+} in uridine uptake are strikingly illustrated when both are omitted from the medium for a few hours, then one or the other is restored. The omission of both for 3 hr causes decrease in uptake to about 10% of the original value, and the shape of the uptake curve indicates that the effect is mainly due to inhibition of uridine phosphorylation (Fig. 1). The restoration of Ca^{2+} alone to such cultures has no stimulatory effect whatever, whereas Mg^{2+} alone restores the full capacity for uptake into the acid-soluble fraction. We conclude from this that Ca^{2+} plays no direct role in regulating the uptake of uridine and that the inhibitory effect of omitting both Ca^{2+} and Mg^{2+} is produced by facilitating the loss of Mg^{2+} from the cells.

In medium containing no added Ca^{2+} and the physiological concentration of Mg^{2+}, there is a slight inhibition of uridine uptake at 3 hr relative to control cultures (Figs. 3 and 4). Although there is about a 10% loss of Mg^{2+} from the cell under these conditions, it cannot by itself account for the inhibition of uridine uptake, since the omission of Mg^{2+} alone causes an equally slight loss of Mg^{2+} from the cell without inhibiting uridine uptake. This suggests that Ca^{2+} omission also causes a shift of Mg^{2+} away from ATP, one of the substrates for uridine kinase, to other binding sites in the cell. A similar conclusion was reached in studies of the effects of both ions on the initiation of DNA synthesis (4, 8). The small decrease in availability of Mg^{2+} due to the omission of Ca^{2+} alone produces a marked decrease in the number of cells synthesizing DNA, with little effect on the uptake of uridine. This difference in response is consistent with the observation (Fig. 2) that much more Mg^{2+} is required to maximize the number of cells synthesizing DNA than to maximize the rate of uridine uptake. However, a moderate reduction of $[Mg_i^{2+}]$ markedly inhibits uridine uptake, as illustrated in Fig. 3 by the drop in uptake to 10% of the original value with a decrease in $[Mg_i^{2+}]$ of merely 50%.

Uridine uptake is an integral component of the coordinate response of chicken embryo cells (5, 8, 10) and 3T3 cells (11, 17) to external effectors. It invariably accompanies other reactions of the coordinate response even when such relatively simple effectors as insulin (18, 19) or excess zinc (18) are used with chicken embryo cells, and Ca pyrophosphate complexes are used with BALB/c3T3 cells (unpublished data). This suggests that the same "second message" that stimulates uridine uptake also stimulates other reactions of the coordinate response such as the initiation of DNA synthesis. Of course the latter reaction is not manifest for several hours after application of the stimulus and is obviously an indirect effect. The evidence offered in the present paper suggests that the availability of Mg^{2+} modulates the uptake of uridine and that serious consideration must be given to its mediation of the other reactions of the coordinate response.

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1. Balk, S. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 271–275.
2. Whitfield, J., MacManus, J., Rixon, R., Boynton, A., Youdale, T. & Swierenga, S. (1976) *In Vitro* **12,** 1–18.
3. Rubin, H. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3551–3555.
4. Rubin, H. & Koide, T. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 168–172.
5. Rubin, H. (1976) *J. Cell. Physiol.* **89,** 613–625.
6. Rubin, H. (1977) *J. Cell. Physiol.* **91,** 449–458.
7. Rubin, A. H. & Chu, B. (1978) *J. Cell. Physiol.* **94,** 13–20.
8. Rubin, A. H., Terasaki, M. & Sanui, H. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4379–4383.
9. 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. 801–816.
10. Bowen-Pope, D. & Rubin, H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1585–1589.
11. Rozengurt, E., Stein, W. & Wigglesworth, N. (1977) *Nature (London)* **267,** 442–444.
12. Martin, T. & Tashjian, A. (1978) *J. Biol. Chem.* **253,** 106–115.
13. Plagemann, P., Marz, R. & Wohlheuter, R. (1978) *J. Cell. Physiol.* **97,** 49–72.
14. Skold, O. (1960) *J. Biol. Chem.* **235,** 3273–3279.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
16. Sanui, H. & Rubin, H. (1977) *J. Cell. Physiol.* **92,** 23–32.
17. Rozengurt, E., Mierzejewski, K. & Wigglesworth, N. (1978) *J. Cell. Physiol.* **97,** 241–252.
18. Rubin, H. & Koide, T. (1975) *J. Cell. Physiol.* **86,** 47–58.
19. Rubin, H. (1977) *J. Cell. Physiol.* **91,** 249–260.