Proc. Nat. Acad. Sci. USA
Vol. 73, No. 1, pp. 168–172, January 1976
Cell Biology

Mutual potentiation by magnesium and calcium of growth in animal cells

(growth regulation/metabolic regulation/divalent cations/coordinate control)

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Communicated by Robley C. Williams, October 24, 1975

ABSTRACT **The effects on DNA synthesis of various combinations of Mg^{2+} and Ca^{2+} in cultures of chick embryo cells have been studied. When $[Mg^{2+}] \geq 0.24$ mM, reduction of Ca^{2+} from the standard concentration of 1.72 mM to 0.01 mM had no effect on the incorporation of [^{3}H]thymidine ([^{3}H]dThd) into DNA over a 16-hr period. When Mg^{2+} was reduced to 0.04 mM, [^{3}H]dThd incorporation into DNA decreased directly with $[Ca^{2+}]$ below 1.72 mM and increased slightly up to $[Ca^{2+}] = 5.02$ mM, where cell damage began to occur. The change in $[Ca^{2+}]$ necessary to maintain a half-maximal rate of [^{3}H]dThd incorporation was found to depend inversely on the fourth power of the change in $[Mg^{2+}]$. Chelation of Ca^{2+} with approximately equimolar ethylene glycol-*bis*(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) in the presence of $[Mg^{2+}] \geq 0.24$ mM reduced [^{3}H]dThd incorporation about 10-fold, and large excesses of EGTA did not further reduce it. The amount of EGTA required to produce a detectable inhibition of [^{3}H]dThd incorporation was independent of $[Mg^{2+}] \geq 0.24$ mM, as was the level of residual incorporation in excess EGTA. When $[Mg^{2+}]$ was reduced to 0.04 mM, however, [^{3}H]dThd incorporation declined even when [EGTA] < $[Ca^{2+}]$, and vanished when EGTA was in large excess. The results are discussed within the framework of a model for the regulation of cell metabolism and growth in which the availability of free Mg^{2+} is the central coordinating factor. The metabolic effects of Mg^{2+} depend on its distribution between elements such as ATP and binding sites on membranes. We propose that the major metabolic effects of varying $[Ca^{2+}]$ are produced indirectly through its competition with Mg^{2+} for membrane sites, thereby making more or less Mg^{2+} available for rate-limiting transphosphorylation reactions.**

The rate of progress of cultured mouse thymocytes into the S-period of the cell cycle is dependent on the concentration of Ca^{2+} in the medium (1). Chick embryo fibroblasts (CEF) also require Ca^{2+} to maintain high rates of DNA synthesis, but in amounts so small that a chelating agent has to be used to eliminate contaminating traces of the cation before inhibitory effects can be detected (2). CEF transformed by infection with Rous sarcoma virus are reported to be unaffected by the removal of Ca^{2+}, and this has been interpreted to mean that Ca^{2+} is not an essential requirement for the initiation of DNA synthesis, but that it plays a regulatory role in nontransformed cells (2). In support of this thesis is the recent finding that DNA synthesis in quiescent 3T3 cultures can be stimulated by adding Ca^{2+} in great excess over the physiological concentration (3).

The investigations of the regulatory role of Ca^{2+} have been restricted to measurements of DNA synthesis, mitosis, and cell number. However, factors such as serum concentration, pH, and population density, which regulate cell proliferation, also regulate, coordinately, many independent metabolic pathways (4). In fibroblasts these include the metabolism of hexoses (5, 6), RNA and protein synthesis (7), and the execution of the differentiated fibroblast functions of hyaluronic acid (8) and collagen production (9). Ca^{2+} is not known to play a significant role in these metabolic pathways (10), and any involvement it may have in their coordinate control is probably indirect.

Mg^{2+} is directly involved in all the regulated metabolic pathways, since it is required for the crucial transphosphorylation reactions (10). In glycolysis, the most fully studied of the pathways, the transphosphorylation reactions are precisely the ones that regulate the flow of glucose to pyruvate (10). They have been shown to be activated by serum (6) and by virus-induced transformation (11). It has been shown that limiting the external supply of Mg^{2+} causes reductions in the rates of DNA, RNA, and protein synthesis, glycolysis, and the transport of 2-deoxy-D-glucose, reductions similar to those observed after removing serum or increasing population density (12). The intracellular availability of free Mg^{2+} has been proposed as the central factor in the coordinate control of metabolism and growth in animal cells (12), with the effects produced by varying the external $[Ca^{2+}]$ possibly arising from the relation between $[Ca^{2+}]$ and the availability of free Mg^{2+} within the cell.

We now report an investigation of the related effects of Mg^{2+} and Ca^{2+} on CEF. We find that reduction of $[Ca^{2+}]$ from the medium to the 0.01–0.02 mM Ca^{2+} that occurs as a contaminant of the other ingredients of the growth medium, has no effect on DNA synthesis as long as $[Mg^{2+}] \geq 0.24$ mM. When $[Mg^{2+}] < 0.24$ mM, inhibition of DNA synthesis occurs, and the inhibition is accentuated by reducing the concentration of Ca^{2+} below physiological levels. From these and other observations, such as the limited inhibition produced by virtually complete removal of free Ca^{2+} with ethylene glycol-*bis*(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA), we conclude that the metabolic effects of varying $[Ca^{2+}]$ are the indirect result of its effect on the availability of free Mg^{2+}.

MATERIALS AND METHODS

The materials and methods used for the culture of CEF, manipulation of divalent cations, and isotopic labeling of cultures were those previously published (12). In most experiments, Mallinckrodt analytical reagent grade $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2H_2O$ were used as the sources of Mg^{2+} and Ca^{2+}. For some experiments Specpure $MgSO_4 \cdot 7H_2O$ and Specpure $CaCO_3$ were obtained from Johnson-Matthey, London, England. EGTA was from Sigma. The medium used was 199 plus 2% tryptose phosphate broth and either 5% dialyzed calf serum or 1% chicken serum, as indicated. When either Mg^{2+} or Ca^{2+} was not deliberately added to the medium the concentrations due to contamination from

Abbreviations: EGTA, ethylene glycol-*bis*(β-aminoethyl ether)-*N,N'*-tetraacetic acid; CEF, chick embryo fibroblasts.

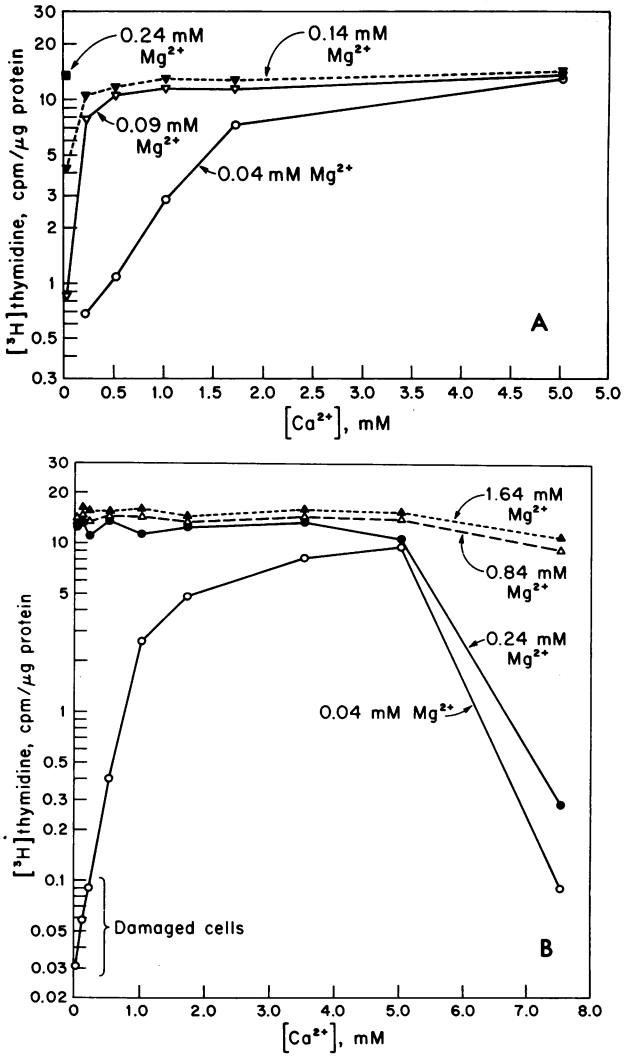

FIG. 1. Effects of various $[Ca^{2+}]$ on [^{3}H]dThd incorporation in the presence of three (A) or four (B) concentrations of Mg^{2+}. Three-day-old cultures of CEF were switched to medium containing 2% tryptose phosphate broth, 5% dialyzed calf serum, and the indicated concentrations of Ca^{2+} and Mg^{2+}. After 16 hr of incubation, the cells were labeled for 1 hr with 0.25 μCi/ml of [^{3}H]dThd and extracted with 0.1 M NaOH. Scintillation counting and protein determinations were done as described (12).

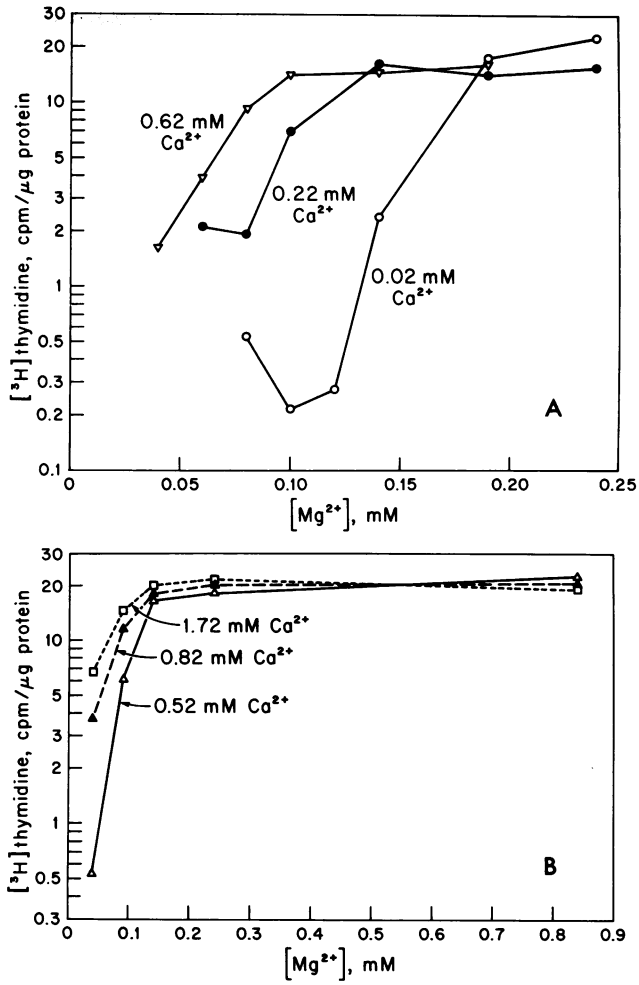

FIG. 2. Effects of various $[Mg^{2+}]$ on [^{3}H]dThd incorporation in the presence of low (A) to moderate (B) concentrations of Ca^{2+}. Procedure was as in legend of Fig. 1. Specpure Mg^{2+} and Ca^{2+} were used in experiment of panel B.

other ingredients were 0.04 mM and 0.02 mM, respectively. In one experiment in which Ca^{2+} and tryptose phosphate were omitted, the concentration of Ca^{2+} was 0.01 mM. The standard medium concentrations were 0.84 mM Mg^{2+} and 1.72 mM Ca^{2+}; standard medium 199 was invariably used during the 1-hr period of labeling cells with [^{3}H]dThd. $[Mg^{2+}]$ and $[Ca^{2+}]$ were measured with a Perkin-Elmer model 403 atomic absorption spectrophotometer.

RESULTS

Figs. 1 and 2 represent four experiments in which the concentrations of both Mg^{2+} and Ca^{2+} were varied, and the effects on the incorporation of [^{3}H]thymidine ([^{3}H]dThd) into DNA determined at 16 hr. When $[Mg^{2+}] \geq 0.24$ mM, the incorporation of [^{3}H]dThd was independent of $[Ca^{2+}]$ in the range 0.02–8.02 mM. When $[Mg^{2+}]$ was decreased below 0.24 mM, increasing amounts of Ca^{2+} were required to maintain high rates of [^{3}H]dThd incorporation. For example, in 0.04 mM Mg^{2+}, the incorporation of [^{3}H]dThd increased with $[Ca^{2+}]$ up to 5.02 mM of the latter (Fig. 1). Still higher concentrations of Ca^{2+} caused precipitate formation in low $[Mg^{2+}]$, with attendant cell damage, and markedly reduced [^{3}H]dThd incorporation. The precipitate as well as the inhibitory effects of very high $[Ca^{2+}]$ were reduced with increased $[Mg^{2+}]$.

Very low concentrations of both Ca^{2+} and Mg^{2+}, which inhibited overall [^{3}H]dThd incorporation to less than $1/10$ that of the standard control in 16 hr, caused cell damage which could not be reversed by restoring standard concentrations of the cations. Such drastic inhibition was due only in part to a reduction in the fraction of labeled nuclei (Table 1); the rest was due to a decreased rate of incorporation of [^{3}H]dThd in cells in the S period and was evident in a 5- to 10-fold reduction in the density of grains above the labeled nuclei (data not shown). Slightly higher concentrations of Ca^{2+} and Mg^{2+}, which inhibited overall incorporation of [^{3}H]dThd to $1/4$–$1/7$ that of the standard control, permitted full recovery of the cells within a day of restoring standard cation concentrations. Such moderate inhibition was due mainly to a reduction in the fraction of labeled nuclei (Table 1), with little reduction in grain density.

The use of complexing agents makes it possible to reduce the level of the free cations far below the minimum attain-

Table 1. Scintillation counting of extracts compared to autoradiography of cells to measure [^{3}H]dThd incorporation F [Ca^{2+}, Mg^{2+}]

Ca^{2+} (mM)	Mg^{2+} (mM)	Scintillation counting	Labeled nuclei
0.02	0.09	0.005*	0.058†
0.02	0.14	0.015	0.155
0.02	0.84	0.67	1.20
0.22	0.04	0.04	0.18
0.22	0.09	0.14	0.26
0.22	0.14	0.78	1.10
0.22	0.84	1.03	0.94
1.72	0.04	0.24	0.34
1.72	0.09	0.70	1.02
1.72	0.14	0.92	0.92
1.72	0.84	1.0	1.0

Cultures were exposed to the indicated concentrations of Ca^{2+} and Mg^{2+} in medium with 2% tryptose phosphate broth and 5% dialyzed calf serum. After 16 hr of incubation, the cultures were labeled for 1 hr with 0.25 μCi/ml of [^{3}H]dThd for extraction and scintillation counting as in Fig. 1, or with both 0.1 and 1.0 μCi/ml of [^{3}H]dThd for autoradiographic studies. The latter were processed (12) and the fraction of labeled nuclei determined.

* Ratio of cpm/μg of protein in experimental to cpm/μg of protein in 1.72 mM Ca^{2+}, 0.84 mM Mg^{2+}.

† Ratio of fraction of labeled nuclei in experimental to fraction of labeled nuclei in 1.72 mM Ca^{2+}, 0.84 mM Mg^{2+}.

able by their deliberate omission from the medium in which they occur as contaminants of the other ingredients. The complexing agents also buffer the medium against changes in free cation concentrations used by their leakage from cells. EGTA exhibits a moderately high affinity for Ca^{2+}, but a very low affinity for Mg^{2+} at the physiological pH of 7.4. When EGTA was added in increasing concentrations to standard medium, it had no effect on [^{3}H]dThd incorporation until it approximately equalled [Ca^{2+}] (Fig. 3A). With small further increments in EGTA, the rate of [^{3}H]dThd incorporation dropped abruptly to about 1/10 that of the control, but dropped no further despite large increases in [EGTA] which reduced free [Ca^{2+}] practically to zero (Fig. 3A).

Varying [Mg^{2+}] from 0.24 mM to 3.24 mM had no effect on the inhibition produced by EGTA. Reduction of [Mg^{2+}] to 0.04 mM, however, which itself reduced [^{3}H]dThd incorporation threefold, sensitized the culture to the reduction in [Ca^{2+}] caused by EGTA. Thus, a further fourfold drop in [^{3}H]dThd incorporation occurred when [EGTA] = 1.6 mM. When [EGTA] was increased beyond [Ca^{2+}], [^{3}H]dThd incorporation fell progressively to insignificantly low levels, and the cells were irreversibly damaged.

Comparison of Figs. 3A and 3B shows that in 0.04 mM Mg^{2+}, less EGTA was required to inhibit [^{3}H]dThd incorporation in the presence of 5% calf serum than in 1% chicken serum. In the presence of the standard [Ca^{2+}], the abruptness of the decrease in [^{3}H]dThd incorporation with relatively small increments in [EGTA] beyond that of [Ca^{2+}] made it difficult to determine precisely the minimum concentration of Ca^{2+} required to maintain maximal rates of [^{3}H]dThd incorporation. The problem was overcome by starting with a concentration of Ca^{2+} which, though very low (10 μM), could maintain a maximal rate of [^{3}H]dThd incorporation in the presence of standard [Mg^{2+}]. A graded series of very low EGTA concentrations was then added to

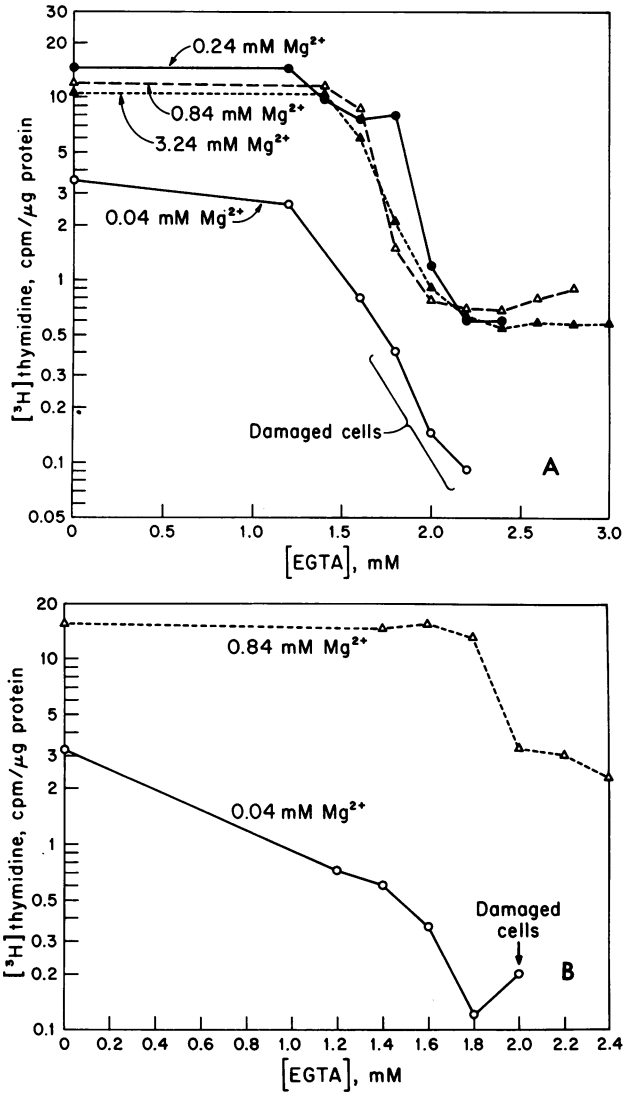

FIG. 3. Effects of various [EGTA] on [^{3}H]dThd incorporation in the presence of four (A) and two (B) concentrations of Mg^{2+}. In the experiment of panel A, 1% chicken serum was used; in that of panel B, 5% dialyzed calf serum was used. Procedure was as in legend of Fig. 1.

gradually reduce [Ca^{2+}] to suboptimal levels. The results of Fig. 4 show a direct relationship between [^{3}H]dThd incorporation and Ca^{2+} in the concentration range of 1–10 μM. As in the previous experiment, addition of EGTA in concentration much greater than Ca^{2+} failed to reduce the rate of [^{3}H]dThd incorporation by more than 10-fold, even though the concentration of free Ca^{2+} approached zero.

The data from eight experiments were collected in Fig. 5, which shows the combinations of [Ca^{2+}] and [Mg^{2+}] required to maintain [^{3}H]dThd incorporation at half of the maximal rate of each experiment. [Ca^{2+}] must be varied inversely as approximately the fourth power of any change in [Mg^{2+}] to maintain half-maximal [^{3}H]dThd incorporation. This suggests that Mg^{2+} is the primary effector and Ca^{2+} a secondary modifier in the regulation of DNA synthesis.

DISCUSSION

The results are most conveniently discussed within the framework of the recently described model for metabolic and growth regulation, in which the availability of divalent

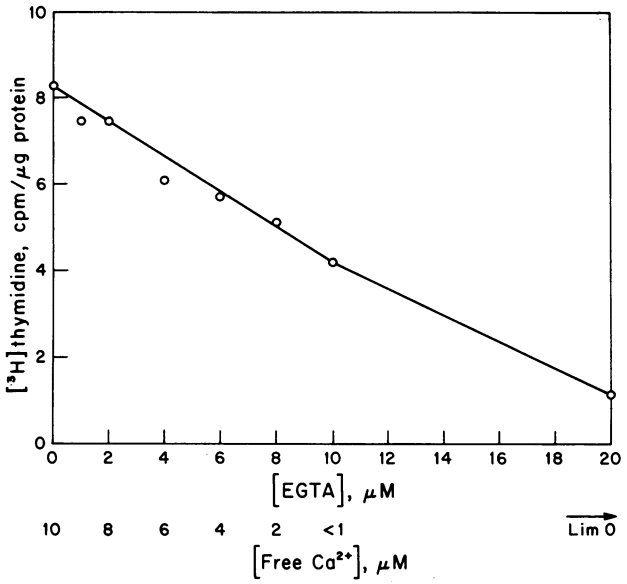

FIG. 4. Dose response of [³H]dThd incorporation to graded, very low concentrations of free Ca^{2+} produced by titration with EGTA. Medium was prepared without tryptose phosphate broth and contained 1% dialyzed chicken serum and 0.84 mM Mg^{2+}. The residual [Ca^{2+}] was 0.01 mM (shown as 10.0 μM). The indicated concentrations of EGTA were added to reduce free Ca^{2+} to the concentrations estimated on the abscissa. The rest of the procedure was as in legend of Fig. 1. One pair of cultures had 1.72 mM Ca^{2+}, which gave the same rate of [³H]dThd incorporation as 0.01 mM Ca^{2+}.

cations within the cell is the principal feature (12). The most important of the divalent cations for metabolism is Mg^{2+}, since it is required for transphosphorylation reactions that are control points in major biochemical pathways (10). Mg^{2+} may occur bound to anionic sites on membranes and macromolecules, complexed with nucleotides and other negatively-charged small molecules, or free in fully ionized form (13). It has been found that only a very small fraction of the Mg^{2+} in cells occurs as the free ion (14, 15). Although the overall concentration of Mg^{2+} in cells is much higher than that of ATP or ADP, only a fraction of the nucleotides is complexed with Mg^{2+}. Since the Mg^{2+}-nucleotide complex is the active substrate in transphosphorylation reactions (10), any variation in free Mg^{2+} would alter the rate of key transphosphorylation reactions. It might also affect ribosomal integrity (16) and tRNA structure (17). That alterations in concentration of free Mg^{2+} occur in physiological control situation is indicated by the increase in the fraction of phosphoglucomutase in the Mg^{2+} form after insulin treatment of muscle cells (18).

Within the context of the model, any agent that displaces Mg^{2+} from membrane sites and makes it available for transphosphorylation reactions or other functions would accelerate a wide range of metabolic processes. Mg^{2+} and Ca^{2+} compete with one another for binding on microsomal membrane preparations (13) and also on plasma membranes (19). On the basis of exchange studies, it is believed that most of the Ca^{2+} in animal cells is associated with surface membranes (20). We assume therefore that the removal of Ca^{2+} from CEF growth medium (2) frees membrane sites for occupation by Mg^{2+}, thus reducing its availability for metabolic reactions. Conversely, the addition of large excesses of Ca^{2+} (3) could free membrane-bound Mg^{2+}, thereby coordinately accelerating many metabolic reactions, and ultimately initiating DNA synthesis. An increase in the rate of DNA synthesis has been observed in the case of 3T3 cells when Ca^{2+} is increased from 1.7 mM to 5 mM (3). While this effect does not take place in the presence of standard [Mg^{2+}] in CEF, it does occur when [Mg^{2+}] is reduced to 0.04 mM (see Fig. 1A and B), although less dramatically than in 3T3 cells. This suggests that 3T3 cells, which are notoriously sensitive to density-dependent inhibition, have a very low intracellular concentration of free Mg^{2+} when crowded.

In view of the intimate relationships between Mg^{2+} and Ca^{2+} in membrane preparations, how can we determine which is acting more proximally in regulating metabolic pathways in whole cells? It should be noted that CEF require much more Mg^{2+} than Ca^{2+} in the medium for optimal functioning, approximately 0.2 mM and 0.01 mM, respectively, when in the presence of standard concentrations of the other. Most of the Mg^{2+} is intracellular, where it is available to participate in metabolic reactions, whereas most of the Ca^{2+} is surface bound (20). There are many Mg^{2+}-dependent reactions in the cell, and these are in many cases

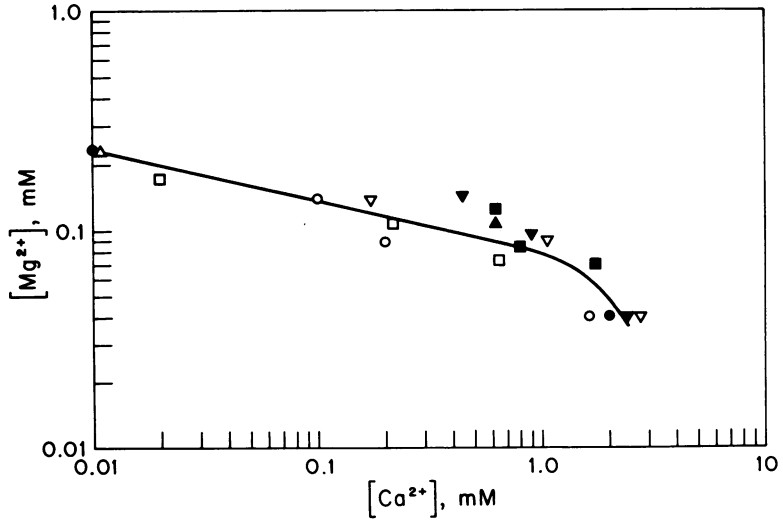

FIG. 5. Combinations of [Ca^{2+}] and [Mg^{2+}] which reduce [³H]dThd incorporation to half the maximum rate of the same experiment. Interpolated values were collected from eight experiments such as those of Figs. 1–4. The different symbols represent different experiments.

known to be control points in metabolic pathways that are responsive to growth regulatory effectors, whereas there are few Ca^{2+}-dependent metabolic reactions in the cell (10). These observations suggest Mg^{2+} as a more likely candidate than Ca^{2+} for providing integrated control of a wide range of metabolic activities, but are hardly conclusive. A more telling finding is the peculiar shape of the curve in Fig. 3A for the inhibition of DNA synthesis by the addition of EGTA in the presence of $Mg^{2+} \geq 0.24$ mM. The entire inhibition occurs within a very narrow range of EGTA concentrations at about the point of equivalence with $[Ca^{2+}]$. The rate of [^{3}H]dThd incorporation is thus reduced about 10-fold, but addition of higher concentrations of EGTA, which must reduce $[Ca^{2+}]$ practically to zero, does not further reduce the rate of [^{3}H]dThd incorporation. In the presence of only 0.04 mM Mg^{2+}, which is itself partly limiting to [^{3}H]dThd incorporation, progressive reduction in $[Ca^{2+}]$ as a result of adding increasing concentrations of EGTA causes a continuous decline in [^{3}H]dThd incorporation (Fig. 3A). By contrast, reduction in $[Mg^{2+}]$ below 0.1 mM leads to a proportional decline in [^{3}H]dThd incorporation in both high and low concentrations of Ca^{2+} (12). These findings can be reconciled by the assumption that the metabolic effects of Ca^{2+} reduction are mediated through the reduced availability of free Mg^{2+}, which has become bound to sites formerly occupied by Ca^{2+}. Since there is much less cellular Ca^{2+} than Mg^{2+} (20), even the total removal of Ca^{2+} would not free enough sites to bind all the Mg^{2+} in the cell. The inhibition produced by removal of Ca^{2+} would therefore be of a limited nature as long as there was sufficient Mg^{2+} present in the medium to maintain a constant concentration of total Mg^{2+} in the cell. Intracellular $[Mg^{2+}]$ remains constant despite wide variations in its extracellular concentration (21), but when this is reduced to extremely low levels by omission of Mg^{2+} from the medium or complexing with pyrophosphate, intracellular levels are reduced (Sanui and Rubin, unpublished). If the supply of Mg^{2+} was already limiting, the removal of membrane-bound Ca^{2+} might be expected to reduce free $[Mg^{2+}]$ to totally ineffective levels so that the rate of [^{3}H]dThd incorporation would approach zero.

The hypothesis that Ca^{2+} removal inhibits [^{3}H]dThd incorporation indirectly through its effect on the availability of Mg^{2+} can explain Balk's discovery that CEF transformed by infection with Rous sarcoma virus are not inhibited by removal of Ca^{2+} (2). We assume that the unregulated growth of the Rous sarcoma cells is caused by an impaired capacity of their membranes to bind divalent cations. This would make large amounts of Mg^{2+} available to maintain high rates of metabolic activity within the cell and the removal of Ca^{2+} from the low affinity membranes would not significantly change the situation. One prediction, now under test, is that removal of Mg^{2+} will inhibit the metabolic activity of those transformed cells that are unaffected by removal of Ca^{2+}.

Attribution of a central role in metabolic regulation in Mg^{2+} does not rule out important functions for other ions in responding to environmental effectors. It seems likely that Ca^{2+} plays some role in cell locomotion (22), control of passive diffusion of ions (23), and intercellular adhesiveness (24). Zn^{2+} has been shown to be involved in nucleic acid synthesis (25), and pH affects all the parameters associated with density-dependent inhibition (26). Only Mg^{2+}, however, has been shown by straightforward biochemical techniques to be directly involved in all the metabolic pathways that constitute the coordinate response of cells to external effectors (10, 26).

Note Added in Proof. Recent experiments show that strontium is almost as efficient as Ca^{2+} in stimulating DNA synthesis in cultures with low $[Mg^{2+}]$. High $[Ca^{2+}]$ in low $[Mg^{2+}]$ causes extensive cell detachment within 2 days. No replacement has been found for Mg^{2+} in supporting normal cell proliferation.

We thank Dr. H. Sanui for teaching us the rudiments of atomic absorption spectrophotometry and critically reviewing this paper. This investigation was supported by NIH Research Grant CA 15744 from the National Cancer Institute.

1. Whitfield, J., Rixon, R., MacManus, J. & Balk, S. (1973) *In Vitro* **8**, 257–278.
2. Balk, S., Whitfield, J., Youdale, T. & Braun, A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 675–679.
3. Dulbecco, R. & Elkington, J. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 1584–1588.
4. Rubin, H. & Fodge, D. (1974) *Control of Proliferation in Animal Cells* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).
5. Sefton, B. & Rubin, H. (1973) *Proc. Nat. Acad. Sci. USA* **68**, 3154–3157.
6. Fodge, D. & Rubin, H. (1973) *Nature New Biol.* **246**, 181–183.
7. Rubin, H. & Koide, T. (1975) *J. Cell. Physiol.* **86**, 47–58.
8. Moscatelli, D. & Rubin, H. (1975) *Nature* **254**, 65–66.
9. Peterkofsky, B. (1972) *Arch. Biochem. Biophys.* **152**, 318–328.
10. Lehninger, A. (1970) *Biochemistry* (Worth Publishers Inc., New York).
11. Singh, V., Singh, M., August, J. & Horecker (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4129–4132.
12. Rubin, H. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 3551–3555.
13. Sanui, H. (1970) *J. Cell. Physiol.* **75**, 361–368.
14. Rose, I. (1968) *Proc. Nat. Acad. Sci. USA* **61**, 1079–1086.
15. England, P., Denton, R. & Randle, P. (1967) *Biochem. J.* **105**, 32C–33C.
16. Meselson, M., Nomura, M. Brenner, S., Davern, C. & Schlessinger, D. (1964) *J. Mol. Biol.* **9**, 696–711.
17. Lindahl, T., Adams, A. & Fresco, J. (1966) *Proc. Nat. Acad. Sci. USA* **55**, 941–948.
18. Peck, E. & Ray, W. (1971) *J. Biol. Chem.* **246**, 1160–1167.
19. Carvalho, A., Sanui, H. & Pace, N. (1963) *J. Cell. Comp. Physiol.* **62**, 311–318.
20. Hickie, R. & Kalant, H. (1966) *Can. J. of Physiol. Pharmacol.* **44**, 893–900.
21. Sparrow, M. (1969) *J. Physiol.* **205**, 19–38.
22. Gail, M. (1973) in *Locomotion in Tissue Cells, Ciba Found. Symp. 14* (Associated Scientific Publishers, Amsterdam), pp. 287–302.
23. Morrill, G. & Robbins, E. (1967) *J. Gen. Physiol.* **50**, 781–792.
24. Steinberg, M., Armstrong, P. & Granger, R. (1973) *J. Membrane Biol.* **13**, 97–128.
25. Rubin, H. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 712–716.
26. Rubin, H. and Koide, T. (1975) *J. Cell. Physiol.* **86**, 47–58.