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Cell Biology

Magnesium and calcium effects on uptake of hexoses and uridine by chick embryo fibroblasts

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

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ABSTRACT **Cultures of chick embryo fibroblasts were incubated for varying periods in media containing different concentrations of Ca^{2+} and Mg^{2+}. Mg^{2+} deprivation produced a gradual decrease in the V_{max} of the glucose transport system for the D-glucose analogues 3-*O*-[^{3}H]methyl-D-glucose and 2-deoxy-D-[^{3}H]glucose and a parallel decrease in the rate of production of lactate from glucose in the medium. It greatly reduced the rates of [^{3}H]uridine uptake and incorporation by decreasing the V_{max} of the uridine transport system. Addition of Mg^{2+} to Mg^{2+}-deprived cultures rapidly increased the rate of [^{3}H]uridine uptake without requiring protein synthesis and increased the rate of 2-deoxy-D-[^{3}H]glucose uptake without requiring RNA synthesis. These effects of changes in Mg^{2+} concentration qualitatively reproduce the effects of such variables as cell density and serum and insulin concentrations. Ca^{2+} deprivation resulted in similar, though much smaller, changes in the activities of the two transport systems, but also greatly increased the "leakiness" of the cells to the nontransported hexose L-[^{3}H]glucose.**

A wide variety of factors, including serum, insulin, cortisol, cell density, pH, and viral transformation, affects the growth of chick embryo fibroblasts in culture (1). The response of the cell to each of these variables consists of a comparable set of changes in a specific array of cellular processes, the best studied of which are: DNA synthesis, uridine uptake into the cell and incorporation into acid-insoluble material, and glucose uptake and metabolism. This relationship between a diverse array of effectors and a single pattern of response has been termed the "coordinate response" (2). Rubin (2) has recently proposed that control of the availability of intracellular Mg^{2+} for crucial Mg^{2+}-requiring cellular reactions is the central mechanism underlying the coordinate response.

Studies in a variety of systems have suggested that changes in the concentration of Mg^{2+} and/or of ATP in the Mg·ATP form have large effects on the activities of individual enzymes (3, 4) and of metabolic pathways (2, 4–6). In our studies, support for Rubin's hypothesis (2) comes from investigations in which the effects of Mg^{2+} and Ca^{2+} concentrations in the culture medium are compared to the effects of cortisol, insulin, and serum (2, 7). In this paper we examine some of the effects of Mg^{2+} deprivation in greater detail and show that the similarity to the effects of serum deprivation or cortisol addition extends to finer levels of analysis.

MATERIALS AND METHODS

Cell Culture. Secondary cultures of chick embryo fibroblasts were prepared in 60-mm culture dishes as described by Rein and Rubin (8). The culture medium used was medium 199 supplemented with 2% tryptose phosphate broth and 1% chicken serum, designated 199 (2-0-1). To investigate the effects of Mg^{2+} and Ca^{2+} concentration, we prepared medium 199 without addition of Mg^{2+} or Ca^{2+} salts but with 2% tryptose phosphate broth and 1% dialyzed chicken serum (2-0-1*d*). This medium contained approximately 0.03 mM Mg^{2+} and 0.02 mM Ca^{2+} (measured by atomic absorption spectrophotometry) and was brought to the desired Mg^{2+} and Ca^{2+} concentrations with added $MgCl_2$ or $CaCl_2$. When a lower concentration of Ca^{2+} was needed (Fig. 2), the medium was passed through Chelex 100 chelating resin (Biorad) in the Na^+ form to reduce Ca^{2+} and Mg^{2+} concentrations to $\leq$ 0.002 mM. $CaCl_2$ and $MgCl_2$ were then added back to achieve the desired concentrations. Except where specifically stated otherwise, the concentration of Ca^{2+} was brought to 0.2 mM.

Measurements with Radioactive Compounds. The following isotopically labeled compounds were purchased from New England Nuclear: 2-deoxy-D-[*G*-^{3}H]glucose ([^{3}H]dGlc), 1.0×10^4 Ci/mol; [5,6-^{3}H]uridine, 4.5×10^4 Ci/mol; L-[*G*-^{3}H]proline, 4.8×10^3 Ci/mol; and 3-*O*-[^{3}H]methyl-D-glucose ([^{3}H]MeGlc) 3.6×10^3 Ci/mol. For measurements at room temperature, cultures were washed once with glucose-free saline buffered at pH 7.4 with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (Hepes), then incubated at room temperature in Hepes-buffered saline of the same ionic composition as the treatment medium and containing the labeled compound at the concentration listed in the figure legend. For measurement at 37°, the radioactive compound was added directly to the treatment medium to give the concentration stated. Uptake was terminated by washing five times with 4 ml of ice-cold glucose-free saline. Unless stated otherwise, all measurements were made with triplicate sister cultures and the averages were plotted.

Production of lactic acid was measured by standard enzymatic methods (9).

RESULTS

Uptake and Incorporation of Uridine. Fig. 1 shows the uptake of 8.84×10^{-8} M ("tracer") and 2×10^{-4} M [^{3}H]uridine into trichloroacetic acid-soluble and -insoluble pools. At 24° the rate of uptake of tracer [^{3}H]uridine is relatively linear with time for 30 min; cultures princubated for 7 hr in 0.028 mM Mg^{2+} take up uridine at 24% of the rate of cultures preincubated in 0.8 mM Mg^{2+}. Uptake of high concentrations (2×10^{-4} M) of [^{3}H]uridine deviates significantly from linearity after as little as 45–90 sec of uptake (see *inset*, Fig. 1*a*), and by 2 hr it seems to be approaching a plateau value. The Mg^{2+}-sufficient cultures contain several times more [^{3}H]uridine at this point than the Mg^{2+}-deprived cultures—a result reminiscent of the finding by Weber and Rubin (10) that rapidly growing cultures accumulate [^{3}H]uridine more rapidly and approach a plateau that is several times higher than that approached by dense, slowly growing cultures.

From Fig. 2 it can be seen that Mg^{2+} and Ca^{2+} deprivation affect [^{3}H]uridine uptake in the same way that reduction in pH

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid.

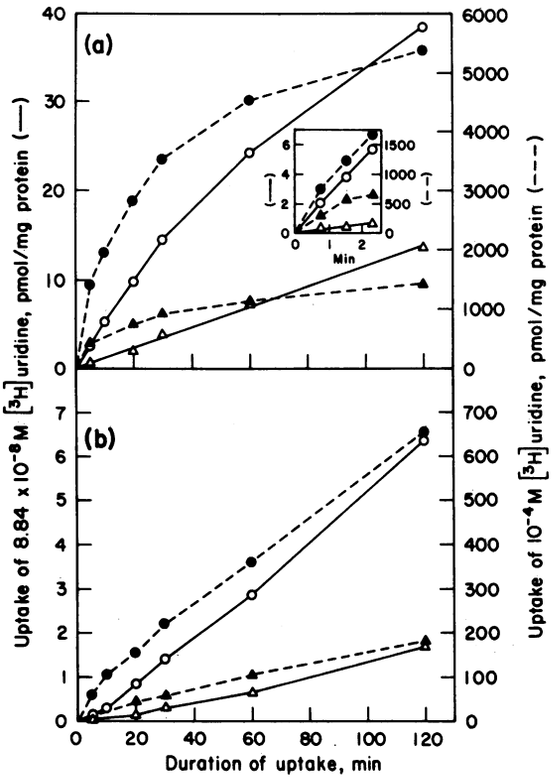

FIG. 1. Uridine uptake and incorporation kinetics. Subconfluent cultures were incubated in medium 199 (2-0-1*d*) containing 0.8 mM Mg^{2+} (○,●) or 0.027 mM Mg^{2+} (△,▲). After 7 hr the medium was changed to Hepes-buffered medium of the same ionic composition containing [^{3}H]uridine at 8.84×10^{-8} M (—) or 1×10^{-4} M (- - - -) and incubated at 24°. After the uptake periods shown on the abscissa, duplicate sister cultures were washed and analyzed for trichloroacetic acid-soluble (*a*) and -insoluble (*b*) intracellular [^{3}H]uridine (*Materials and Methods*). *Inset* in (*a*) shows data from a similar experiment in which shorter periods of uptake were measured using triplicate sister cultures. SD < 10%.

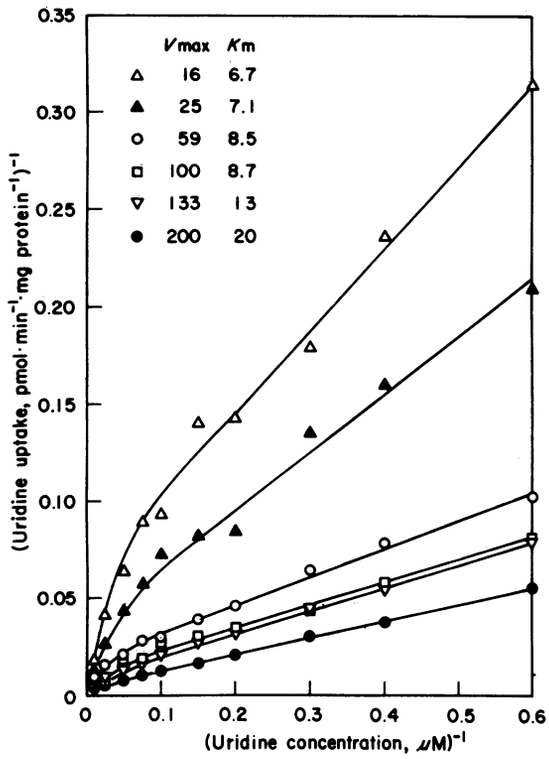

FIG. 2. Uridine uptake. Subconfluent cultures were incubated in medium 199 (2-0-1*d*) containing 0.8 mM Mg^{2+} and 0.2 mM Ca^{2+} (control) or in media derived from control. After incubation for the times indicated, rates of uptake of 1.67×10^{-6}–1×10^{-4} M [^{3}H]uridine into total cellular material were determined, using triplicate sister cultures, with a 3-min incubation at 24° in Hepes-buffered medium of the same ionic composition as the treatment medium. SD < 5%, except in the ethylene glycol-bis(β-aminoethylether)-*N,N'*-tetraacetic acid (EGTA)-containing series (□) and in the 6.7-hr low Mg^{2+} series (▲), in which SD ≤ 13%. The data are plotted as a double-reciprocal (Lineweaver-Burk) plot and K_m (μM) and V_{max} (pmol·min^{-1}·mg of protein^{-1}) values were calculated from the linear portions, taken as concentrations ≤ 10^{-5} M (i.e., using values of μM uridine^{-1} ≥ 0.1). (●) Control, 4.5-hr incubation. 0.028 mM Mg^{2+}: (▽) 4.5-hr, (▲) 6.7-hr, and (△) 7.5-hr incubation. (□) 0.24 mM EGTA, 7-hr incubation. (○) pH 6.8, no serum, 8.5-hr incubation.

and serum concentration do, largely by decreasing the V_{max} but with a decrease in K_m as well. Weber and Rubin (10) have shown that high cell density produces similar changes in V_{max} and K_m.

With tracer concentrations (8.8×10^{-8} M) of [^{3}H]uridine the magnitude of the effect of Mg^{2+} deprivation on incorporation of label into trichloroacetic acid-insoluble material is identical to the effect on uptake of the label into trichloroacetic acid-soluble material (Fig. 3*a*). A comparable result has been obtained by Weber and Rubin (10) when comparing sparse and dense cultures of chick fibroblasts and by the present investigators with variations in serum, insulin, and cortisol concentration (data not shown). It is possible that these effects on the rate of incorporation of [^{3}H]uridine into trichloroacetic acid-insoluble material do not represent changes in rates of RNA synthesis, but merely reflect changes in the rate of uptake of labeled precursors.

In the double-reciprocal plot in Fig. 2 the points closest to the origin (i.e., at the highest uridine concentrations) are deflected downward from the straight line fitted to the other points. This indicates that at high uridine concentrations more uridine is entering the cell than can be accounted for by a transport system behaving in a simple Michaelis-Menten fashion, and has been attributed (11) to nonmediated uptake. Because the *relative* importance of leakiness is greater in cultures with low facilitated transport activity, the difference in uptake rates between untreated and Mg^{2+}-deprived cultures is smallest at high uridine concentrations.

Because changes in rates of uridine transport induced by culture variables like serum and pH have been shown to be at least partially independent of changes in rates of macromolecular synthesis (7), we looked to see if this were true for changes induced by Mg^{2+} deprivation. Fig. 4*a* shows that changing into a medium containing 0.028 mM Mg^{2+} did not result in a decrease in existing uptake rates until the sixth hour of incubation, although the increase in rate in control cultures, apparently produced by the fresh serum or the medium change itself, did not occur in the Mg^{2+}-deprived cultures. During this period, readdition of Mg^{2+} to Mg^{2+}-deprived cultures permitted a gradual increase in rate comparable to the increase seen in control cultures. After the initial 6-hr period the rate of uridine uptake dropped quickly, reaching 6% of control rate by the end of the experiment (14 hr). Readdition of Mg^{2+} during this period produced a rapid increase to a rate comparable to that which preceded Mg^{2+} deprivation. Cycloheximide at a concentration (1 μg/ml) that reduced [^{3}H]proline incorporation in Mg^{2+}-containing cultures by 91% did not interfere with the rapid increase in transport activity produced by readdition of Mg^{2+} to Mg^{2+}-deprived cultures.

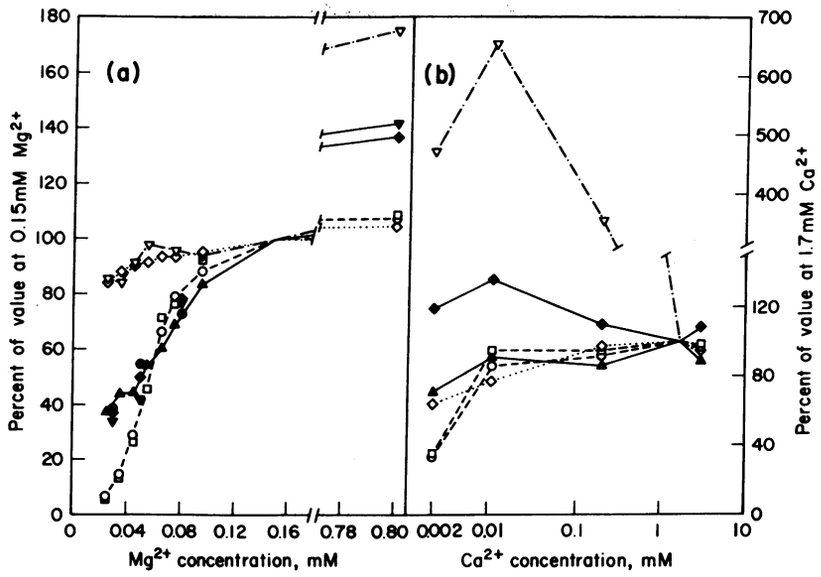

FIG. 3. Mg^{2+} and Ca^{2+} dose response. Subconfluent cultures were incubated in medium 199 (2-0-1*d*) containing 0.2 mM Ca^{2+} and 0.025–0.8 mM Mg^{2+} (*a*) or 0.15 mM Mg^{2+} and 0.002–3 mM Ca^{2+} (*b*). After 8 hr the following measurements were made (*Materials and Methods*). (○) Trichloroacetic acid-soluble [^{3}H]uridine, 28 min at 37°, SD < 10%. (□) Trichloroacetic acid-insoluble [^{3}H]uridine, 28 min at 37°, SD < 10%. (▽) 2.3×10^{-7} M (tracer) L-[^{3}H]Glc, 19½ min at 38°, SD < 10%. (▲) 10^{-7} M (tracer) [^{3}H]dGlc, 28 min at room temperature, SD < 5%. (▼) 5.5 mM [^{3}H]dGlc, 16 min at room temperature SD < 5%. (♦) 5.5 mM [^{3}H]MeGlc:10, 60, and 120 sec at room temperature. Initial rates of uptake were calculated from these points. (●) Rate of production of lactate from 5.5 mM Glc, 2 hr at room temperature, SD of quintuplicate measurements was ≤15%. (◊) mg of protein per dish, SD < 5%.

Uptake of L-[^{3}H]Glc. L-Glc is the enantiomorph of the commonly occurring D-Glc. Since the uptake of L-[^{3}H]Glc is slow (<3% of [^{3}H]dGlc uptake rates at <0.1 mM hexose) and is nonsaturable within a 0.00002–20 mM concentration range (data not shown), it does not seem to enter the cell with the aid of a facilitated transport system. Changes in the rate of L-[^{3}H]Glc permeation can be used as indications of changes in the leakiness of cells to hexoses. Fig. 3 shows that Mg^{2+} removal decreases, and Ca^{2+} removal greatly *increases*, the permeability of cells to L-[^{3}H]Glc, suggesting that Ca^{2+} plays an important role in maintaining the permeability barrier of the cells.

Uptake of [^{3}H]dGlc and [^{3}H]MeGlc. In 0.2 mM Ca^{2+}, decreasing the concentration of Mg^{2+} in the medium below about 0.150 mM has a large effect on the rates of [^{3}H]dGlc and [^{3}H]MeGlc uptake (Fig. 3*a*). Since measurement of [^{3}H]MeGlc uptake does not depend on the ability of the cell to phosphorylate the incoming hexose (11), the agreement between these two measurements supports the hypothesis that we are measuring an effect of [Mg^{2+}] on transport activity, rather than on phosphorylation.

In Fig. 5 we show that the effect of Mg^{2+} deprivation on [^{3}H]dGlc accumulation is not reproduced by experimental reduction of the cells' ability to phosphorylate intracellular hexose. One-hour incubation in 0.05 mM iodoacetate, a potent inhibitor of glyceraldehyde-3-phosphate dehydrogenase, greatly reduces the cellular rate of phosphorylation of [^{3}H]dGlc (measured after separation on an anion exchange column, data not shown) but actually increases the initial rates of [^{3}H]dGlc uptake (and of [^{3}H]MeGlc uptake, data not shown). By 2 min the cells contain approximately the amount of label expected if the [^{3}H]dGlc were in equilibrium between intracellular and extracellular space. Since the glucose transport system of chick embryo fibroblasts does not accumulate hexose against a concentration gradient, the further accumulation of label requires that intracellular [^{3}H]dGlc be trapped by phosphorylation. Thus, in iodoacetate-treated cultures the rate of label accumulation decreases after 2 min. This kinetic pattern is not seen with the control or Mg^{2+}-deprived cultures, or as a result of changes in serum concentration or cell density (data not shown), suggesting that in those cases the phosphorylating capacity of the cell remains adequate to handle [^{3}H]dGlc entering at the rate determined by the activity of the transport system.

In contrast to the results with Mg^{2+} deprivation, rates of [^{3}H]dGlc and [^{3}H]MeGlc uptake are not equally affected by variations in Ca^{2+} concentration (Fig. 3*b*). Ca^{2+} deprivation increases rates of [^{3}H]MeGlc uptake whereas it decreases rates of [^{3}H]dGlc accumulation. Although we have not made a detailed analysis of this phenomenon, we calculate that at low Ca^{2+} concentrations the large increase in the rate of nonmediated hexose uptake ("leakiness") measured by L-[^{3}H]Glc uptake can account quantitatively for much of the increase in [^{3}H]MeGlc uptake rate. [^{3}H]dGlc has a lower K_m than [^{3}H]MeGlc (12) for the glucose transport system, so that at low hexose concentration nonmediated uptake accounts for a smaller proportion of the total uptake. This makes measurement of [^{3}H]dGlc uptake less sensitive to increases in leakiness and more sensitive to effects on the transport system.

Table 1 indicates that deprivation of Mg^{2+}, Ca^{2+}, serum, or insulin, or the addition of cortisol, decreases the rate of [^{3}H]dGlc transport. These decreases result from a lowering of the V_{max} of the transport system with little change in the K_m.

To investigate the involvement of macromolecular synthesis in changes in [^{3}H]dGlc transport rate, we followed the decline in transport activity as a function of time after Mg^{2+} deprivation. In Fig. 4*b* a difference between Mg^{2+}-sufficient and Mg^{2+}-deprived cultures is evident by the first hour, and the rate [^{3}H]dGlc transport in Mg^{2+}-deprived cultures continues to decline until the experiment was terminated at 14 h. Although the activity of the glucose transport system in Mg^{2+}-deprived cultures is not always increased by readdition of Mg^{2+}, even in experiments showing rapid increases in [^{3}H]uridine uptake rates, in this experiment readdition of Mg^{2+} to cultures deprived of Mg^{2+} for 4½, 6¾, or 9¾ hr produced a large increase in transport activity. Thirty-minute preincubatin in 0.1 μg/ml of

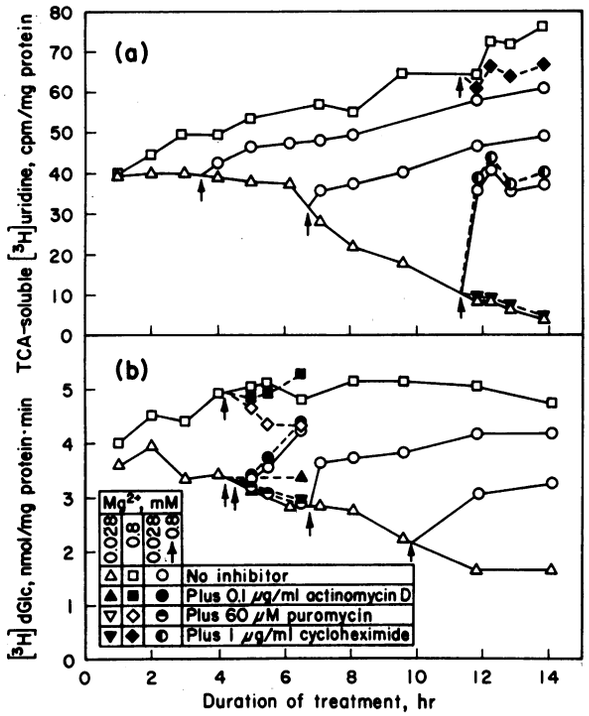

FIG. 4. Time course of effects of Mg^{2+} deprivation. Subconfluent cultures were changed into medium 199 (2-0-1*d*) containing 0.028 mM Mg^{2+} (Mg^{2+}-deprived) or 0.8 mM Mg^{2+} (Mg-sufficient). At the times indicated by the arrows some of the cultures received an inhibitor either of RNA or of protein synthesis, or enough $MgCl_2$ to bring low Mg^{2+} cultures to 0.8 mM Mg^{2+}, or both. Symbols are defined in the inset to (*b*). Parts (*a*) and (*b*) were done on different days. Measurements: (*a*) Uptake (15 min at 24°) of 2.21×10^{-8} M [^{3}H]uridine (1 μCi/ml) into trichloroacetic acid (TCA)-soluble cell contents, SD < 5%. (*b*) Uptake (15 min at 24°) of 10^{-7} M [^{3}H]dGlc (1 μCi/ml) in the presence of 5.5 mM unlabeled glucose, SD < 5%.

actinomycin D reduced [^{3}H]uridine incorporation to 13% of control rate but did not prevent the increase in [^{3}H]dGlc transport rate induced by Mg^{2+} readdition to Mg^{2+}-deprived cultures. To inhibit protein synthesis puromycin was used in place of cycloheximide because cncentrations of cycloheximide sufficient to reduce [^{3}H]proline incorporation to less than 10% of control rates resulted in a very rapid decrease in [^{3}H]dGlc uptake rates (data not shown). In Fig. 4*b* 60 μM puromycin, which reduced [^{3}H]proline incorporation to 5% of control rates, blocked the incrase in [^{3}H]dGlc uptake rate induced by readdition of Mg^{2+} and produced some inhibition of uptake in Mg^{2+}-sufficient cultures.

Incorporation of [^{3}H]Proline. A 7½-hr incubation in low Mg^{2+} medium (0.035 mM) reduces [^{3}H]proline incorporation by about 3-fold (Table 1). The quantity of trichloroacetic acid-soluble [^{3}H]proline within the cell after the 20-min labeling period is only slightly (20%) decreased (data not shown).

To determine whether changes in rates of protein synthesis occurred rapidly enough to be involved in changes in the activity of the glucose transport system, we followed the effects of Mg^{2+} deprivation on [^{3}H]proline incorporation. Proline incorporation decreased rapidly during incubation in low Mg^{2+} medium. Readdition of Mg^{2+} produced a rapid increase in rate of incorporation, though not a complete restoration to pretreatment rate. A concentration of actinomycin D (0.1 μg/ml) that reduced [^{3}H]uridine incorporation to 30% of control in this experiment did not prevent the reversal, though during the next few hours [^{3}H]proline incorporation by control, reversed, and

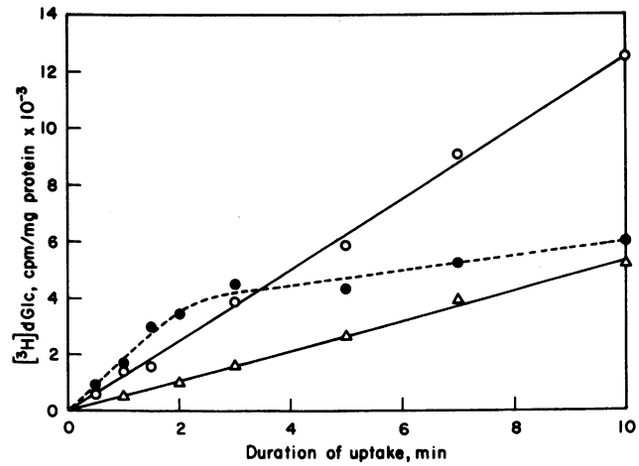

FIG. 5. Effect of glycolytic inhibitor on [^{3}H]dGlc uptake. Subconfluent cultures were incubated for 8 hr in medium 199 (2-0-1*d*) containing 0.028 mM (△) or 0.8 mM (○)Mg^{2+}. Iodoacetate (0.05 mM) was then added to some of the 0.8 mM Mg cultures (●) and 2 hr later the rate of uptake of 1 mM [^{3}H]dGlc in the presence of 2 mM glucose was measured at 24° in Hepes-buffered medium. SD ≤ 5%.

Mg^{2+}-deprived cultures was significantly depressed by the actinomycin D.

DISCUSSION

It is well known that many cellular enzymes, in particular those catalyzing transphosphorylation reactions, require Mg^{2+} for maximal activity *in vitro* (3, 4–6, 13). Rubin (2) has recently proposed that a variety of culture variables alter the availability of intracellular Mg^{2+} to these crucial reactions and in this way regulate the life of the cell. We have attempted to reproduce the effects of culture variables by incubating cultures of chick embryo fibroblasts in medium low in Mg^{2+}. Use of intact cultures permits us to extend the data on the Mg^{2+}-sensitivity of cellular enzymes *in vitro* to enzyme systems that remain in their original cell compartment or structure (for example, by mea-

Table 1. K_m and V_{max} of [^{3}H]dGlc transport

	K_m, mM	V_{max}, nmol/mg protein·min	[^{3}H]Proline incorporation, % value in 1% chick serum
Control	3	8.3	100
− Serum	3	6.3	72
Low Mg^{2+} (0.035 mM)	2.5	4.5	35
Low Ca^{2+} (≤ 0.002 mM)	2.5	7.1	65
− Serum + 10^{-7} M cortisol	3	2.9	47
− Serum + 10^{-7} M cortisol + 0.1 unit/ml insulin	3	14.3	105

Subconfluent cultures were incubated 7½ hr in medium 199 containing 0.8 mM Mg^{2+} and 0.2 mM Ca^{2+} plus 1% chick serum (controls) or in media derived from control as described. Rates of uptake of 0.2, 0.25, 0.33, 0.5, 1.0, and 2.0 mM [^{3}H]dGlc were measured in Hepes-buffered saline of the same composition and K_m and V_{max} values were determined from Lineweaver-Burk plots of the data. [^{3}H]Proline incorporation into trichloroacetic acid-insoluble material was determined after 45 min of uptake at 37°.

suring lactate production), as well as to cell functions, like progression through the cell cycle and the transport of nutrients whose Mg^{2+} dependence cannot be measured *in vitro*.

The systems that transport uridine and glucose in chick embryo fibroblasts are not known to catalyze transphosphorylation reactions or perform other activities that would suggest *a priori* that they would require Mg^{2+} for activity. Nevertheless, our hypothesis led us to predict that, since uridine and glucose transport are affected by culture variables, they would also be sensitive to changes in the availability of Mg^{2+}.

The nature of the effect of Mg^{2+} deprivation on [^{3}H]uridine uptake as measured by changes in K_m and V_{max} (Fig. 2), as well as by characteristics of the kinetics of uptake and incorporation (Fig. 1), is identical to the effect of serum concentration (Fig. 2) and population density (10). Since the decrease in uridine transport rate can be rapidly reversed by Mg^{2+} readdition even if protein synthesis is inhibited (Fig. 4*a*), it is unlikely that Mg^{2+} affects uridine transport only indirectly via effects on protein synthesis.

The effects of Mg^{2+} deprivation on glucose transport and conversion to lactate are also analogous to the effects of culture variables. Mg^{2+} deprivation decreases rates of glucose transport by decreasing the V_{max} of the transport system without change in K_m (Table 1). If one of the glycolytic enzymes known to require Mg^{2+} had proven more sensitive than the glucose transport system to Mg^{2+} deprivation, we would have expected a result similar to that obtained when iodoacetate was used to inhibit glyceraldehyde-3-phosphate dehydrogenase (Fig. 5). In that case, long-term [^{3}H]Glc accumulation and lactate production (data not shown) were inhibited but the activity of the glucose transport system, as measured by short-term [^{3}H]MeGlc and [^{3}H]dGlc uptake, was not. With Mg^{2+} deprivation we find that the activity of the glucose transport system and the rate of lactate production show parallel decreases (Fig. 3*a*). Investigating serum concentration and transformation by Rous sarcoma virus, Bissell *et al.* (14) have demonstrated a comparable parallel change in glucose transport activity and the rate of lactate production.

A complication arises with the relationship between changes in glucose transport activity and macromolecular synthesis. Fig. 4*b* shows that restoration of [^{3}H]dGlc transport activity upon Mg^{2+} readdition is prevented by inhibitors of protein, though not of RNA synthesis. This is in contrast to results with serum stimulation of glucose transport (1), which is independent of protein synthesis during the early stages. It is possible that these difficulties are related to the disproportionately large effects of severe Mg^{2+} deprivation on proline incorporation. Whereas serum removal or cortisol addition rarely results in more than a 2-fold reduction in rates of [^{3}H]proline incorporation (Table 1), lowering [Mg^{2+}] in the medium to 0.028 mM can reduce incorporation by a factor of 3 or more. We suggest that the disproportionately large effect of Mg^{2+} deprivation on proline incorporation is a consequence of our inability to exactly duplicate, by Mg^{2+} deprivation and readdition, the changes in distribution of Mg^{2+} between different cell compartments that are produced by insulin and other culture variables.

The data presented here on Ca^{2+} deprivation suggest that Ca^{2+} is required to maintain the cell permeability barrier (15). Ca^{2+} deprivation greatly increases the "leakiness" of the cell membrane, as measured by increased L-[^{3}H]Glc influx rates, and increases [^{3}H]MeGlc uptake rate, which we interpret as a consequence of this increased leakiness. Ca^{2+} deprivation inhibits the cellular activities sensitive to Mg^{2+} deprivation but the effect is smaller, even with virtually complete removal of Ca^{2+} ([Ca^{2+}] < 0.002 mM) from the media. For the Mg^{2+} deprivation experiments described in this paper we have made use of the interaction between Ca^{2+} and Mg^{2+} in the medium by reducing the concentration of Ca^{2+} found in normal growth medium (1.7 mM) to 0.2 mM. This increases the sensitivity of the cells to Mg^{2+} deprivation and allows us to obtain reproducible results without resorting to complexing agents (16). Rubin and Koide (16) have suggested that the effects of Ca^{2+} deprivation may result from changes in the distribution of intracellular Mg^{2+} brought about by a change in the pattern of competition between Ca^{2+} and Mg^{2+} for intracellular binding sites. It is also possible (3) that changes in intracellular [Ca^{2+}] are involved directly in regulation of metabolic activity, though only $1/10$ as many enzymes require Ca^{2+} as Mg^{2+} (17). For the results reported here, however, it is clear that variations in Mg^{2+} concentration more faithfully reproduce the effects of culture variables than do variations in Ca^{2+} concentration.

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