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Regulation of Glucose Carriers in Chick Fibroblasts

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The derepression of glucose transport initiated by removing glucose from the incubation medium requires both protein and RNA synthesis. The synthesis and accumulation of putative mRNA for the carrier protein(s) can be demonstrated by inhibiting protein synthesis with cycloheximide (2 μg/ml). Release from inhibition with simultaneous addition of actinomycin D (1–5 μg/ml) results in a burst of carrier synthesis that achieves virtually maximal derepression in 4–6 h. An external energy source provided by a "nonrepressive" sugar (D-fructose, D-xylose) or by pyruvate is required to accomplish carrier synthesis. Previous failure to demonstrate mRNA accumulation was due to the depletion of energy in the starved cells. Glucose acts as a repressor at a posttranscriptional step, probably at the level of turnover of formed carrier.

The protection of formed carrier in the absence of glucose and by inhibitors of protein synthesis even in the presence of glucose has encouraged conjecture that a protease is activated by a metabolic product of glucose that is analogous to a co-repressor. The glucose metabolite either activates the protease by direct interaction with it or alters the conformation of the carrier to expose a critical region to protease attack. Indeed the regulation of carrier density in the membrane of chick fibroblasts may be achieved entirely by carrier inactivation, the rate of which is a function of glucose concentration in the culture medium.

Key words: glucose, carrier, regulation, transport

The derepression of glucose transport that follows when cultivated mammalian and avian cells are deprived of a carbon source, has provided a means of examining the mechanisms controlling hexose carrier synthesis and turnover (1–7). The most detailed analysis of elements involved in determining carrier concentration has been contributed by Christopher and Kalckar (8, 9). They have proposed that regulation involves the turnover of components of hexose uptake systems on the one hand and hexose carrier synthesis which may or may not be alternatively accelerated and restrained on the other.

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Since the phenomenon of "deprivation derepression" first described in glucose transport by chick fibroblasts (1) has now been extended to amino acid transport in mammalian cells (10) and to the leucine-binding protein of E. coli (11), some aspects of the mechanisms involved may have a broader interest than that of hexose transport in vertebrate cells.

In chick fibroblasts (1, 6, 7, 12, 13) and in Nil (hamster) fibroblasts (8, 9) the increase in carrier molecules during starvation is blocked at virtually any stage by inhibitors of protein synthesis (cycloheximide and puromycin) as well as by inhibitors of RNA synthesis (actinomycin D and cordycepin). The inhibition of protein synthesis is readily explained as blocking the formation of new carriers while carrier destruction proceeds at some finite rate. Why inhibitors of RNA synthesis are effective after derepression is initiated has been difficult to reconcile with the commonly observed features of the synthesis of protein. Generally when mRNA molecules are permitted to accumulate (14, 15), translation proceeds and is unaffected by the later addition of inhibitors of RNA synthesis. Indeed the so-called "paradoxical effect" of actinomycin D is often observed (16).

Several observations with respect to repression and derepression of glucose transport await a more general hypothesis to be explained. Among these are the derepression that is characteristic of cells cultivated on D-galactose, D-fructose, and D-xylose as sole carbon source. Certain glucose analogues such as 2-deoxy-D-glucose and D-glucosamine simulate glucose as "repressors" of transport in repressed cells but have no "repressor" effect when added to derepressed cells. Glucose, on the other hand, effects a sharp repression of derepressed cells (8, 9, 13).

In the course of studies of ATP conservation by normal cell lines and early passage cell isolates, we have rediscovered the dramatic lowering of the ATP level in a variety of mammalian and avian cells by 2-deoxy-D-glucose and D-glucosamine (30).

This led to a series of experiments that provided presumptive evidence for the synthesis and accumulation of putative mRNA for hexose carrier protein. In addition a posttranscriptional negative control by glucose emerged. This last provides additional evidence for the Christopher-Kalckar model of hexose carrier turnover (8, 9).

MATERIALS AND METHODS

Cell Cultures

Both primary and secondary chick embryo fibroblast (CEF) cultures were prepared by trypsinization as previously described (12). Cells were grown in small (30–50 cm^2) glass bottles, T-flasks, roller bottles, or multiwell plates. The medium employed in all experiments was Eagle's basal medium (BME) (19) without $NaHCO_3$ and supplemented with 3% or 4% calf serum. The cultures were incubated in sealed vessels in incubators not gassed with CO_2. This lack of $NaHCO_3$ and CO_2 is critical to the growth and assay of glucose uptake in chick cells (20) in that the depression observed in starved cells is far greater when cells are maintained in the absence of $NAHCO_3$ and CO_2.

Both primary and secondary chick cells were inoculated into multiwell plates at approximately 2–3 $\times$ 10^4 cells per well. At 3–4 days as the cells were approaching confluence, they were used for the experiments described.

Measurement of D-glucose Entry into Cells

a. Bottles and T-flasks. The following procedures were conducted at 37°C with reagents equilibrated at 37°C. Monolayers were washed twice with 20 ml of balanced salt

solution (BSS) without glucose or with normal saline. D-[^{3}H] Glucose (2 μC/ml, 4×10^{-6} M) or D-[U-^{14}C] glucose (0.5 μC/ml, 4×10^{-6} M) was added in 1.0 ml BSS Glc^- (glucose-free) and the monolayers were incubated on a rotatory shaker (80 oscillations per minute) for 5 min. The reaction was stopped by washing twice with 40 ml of normal saline. After draining, the cells were covered with 1.5 ml cold 5% trichloroacetic acid (TCA) and allowed to stand 15–25 h at 4°C. The TCA solution was transferred quantitatively into scintillation vials and neutralized with NaOH. Samples were counted in a liquid scintillation counter after addition of 15 ml of Bray's scintillation fluid. The protein content of each cell population (which remained fixed to the culture vessel after exposure to 5% TCA) was measured by the method of Lowry et al. (21). The acid-insoluble fraction, as reported previously (1), contained less than 1% of the total cell-associated counts.

b. Multiwell plates. Sugar Uptake Assays. At the end of the "preconditioning" period, the monolayers were washed by rinsing them twice with BSS Glc^- and chilled on ice, whereupon 0.3 ml of ice cold radiolabeled sugar solution was added per well. The plates were allowed to float on a 37°C water bath for the pulse period (usually 5 min) desired.

The uptake was stopped in one motion by immersing the plate in a beaker containing cold PBS. After draining, 0.5 ml of 5% TCA was added to each well and the plates were kept at 4°C overnight. The TCA-soluble phase was obtained, mixed with 10 ml scintillation fluid, and counted on a Beckman LS-230 scintillation counter.

Materials. [U-^{14}C] Glucose 196 mCi/mmole; D-[2-^{3}H] glucose 540 mCi/mmole; D-[3-^{3}H] glucose 5 Ci/mmole; [^{3}H] 3-O-methyl glucose (140 mCi/ mmole); D-glucose-6-[^{3}H] amine 10 Ci/mmole; and D-[1-^{14}C] mannose 50 mCi/mmole were purchased from New England Nuclear Corporation (Boston, Massachusetts); D-glucose, D-galactose, and D-fructose were obtained from Sigma Chemical Company (St. Louis, Missouri); 2-deoxy-D-glucose and 3-O-Methyl glucose from Calbiochem (La Jolla, California); and 6-deoxy-D-glucose from Schwarz-Mann (Orangeburg, New York). Bovine insulin was purchased from Schwarz-Mann, Calbiochem, and Sigma Chemical Company, dissolved in dilute acid (0.01 N HCl) at a concentration of 10 units per milliliter, sterilized by filtration through Millipore filters, and neutralized upon dilution before use. Cycloheximide, actinomycin D, and cordycepin were purchased from Sigma Chemical Company. Purified yeast hexokinase was obtained from Sigma Chemical Company and from Schwarz-Mann.

RESULTS AND DISCUSSION

Deprivation Derepression of Glucose Transport

When CEF cells growing on D-glucose are washed and resuspended in complete BME with or without dialyzed serum and without glucose their capacity to transport glucose, 2-deoxyglucose and 3-O-methyl glucose (1, 17, 12) is progressively increased. Within 24 h of incubation at 37°C such cells can transport glucose and certain of its analogues 15 to 25 times more rapidly than at the start of the starvation. If glucose is included in the medium at the usual concentration (5.5 mM), the derepression of glucose uptake is not observed and at the end of 24 h the rate of transport is little changed from that at the start (1). At equimolar concentrations, 2-deoxy-D-glucose (2DOG), D-mannose, and D-glucosamine are about equally effective in maintaining the repressed level of glucose transport (1, 13).

Growth or maintenance on certain other naturally occurring sugars results in derepression equal to or surpassing that induced by starvation. Among the sugars of the latter category are D-fructose and D-xylose (1, 8, 9, 22). D-Galactose at times resembles D-glucose at concentrations of 5.5 mM or higher and at others permits maximal derepression. At concentrations lower than 5.5 mM the repressive effect of several sugars on glucose transport is reduced or is not exerted at all. Such is the case for 2DOG, D-glucosamine, D-galactose, and D-mannose. This is true of cells grown either in medium containing serum or in serum-free medium. Glucose on the other hand remains an effective repressor and even concentrations as low as 0.055 mM have considerable repressive action over a 24-h period (13) (Fig. 1).

Asymmetry of Repression of Transport of Different Hexoses by the Same Treatment

Somewhat surprisingly (13) the hexoses previously thought to use the same carrier as glucose are not equally derepressed by the same pretreatment of the cells. It should be pointed out that the differential contribution of phosphorylation by hexokinase, glucokinase, galactokinase, and fructokinase to what is deemed "uptake" is far from clear. Nonetheless while starvation derepresses glucose uptake 30-fold, 2DOG will be derepressed somewhat less (20-fold), followed in descending order by mannose (15-fold), galactose (5–7-fold), and glucosamine (2-fold). Glucose represses the uptake of all 5 sugars effectively at a concentration of 5.5 mM (Fig. 2). A 10-fold reduction in glucose concentration in the conditioning medium remains repressive for glucose uptake, but mannose uptake escapes repression. Glucosamine is a good repressor of glucose transport, but mannose transport is derepressed at the same glucosamine concentration. Thus while it is not clear what

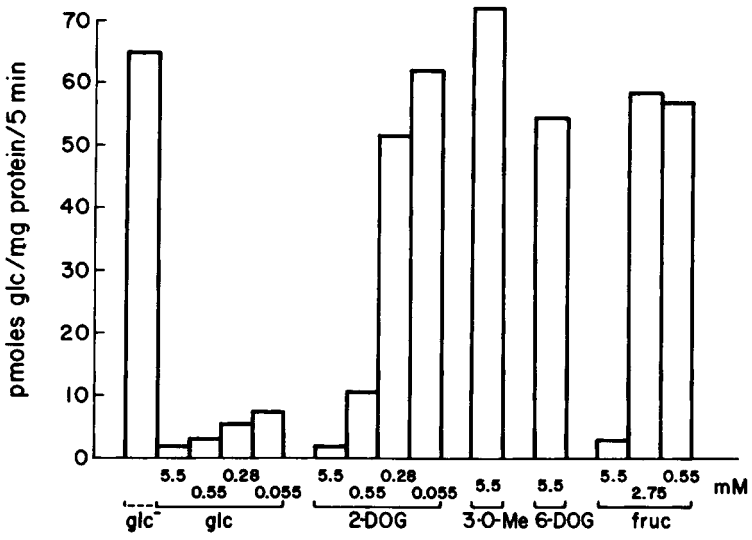

Fig. 1. Pattern of derepression of glucose uptake as a function of the concentration of carbon source. Chick embryo fibroblasts (CEF) were seeded at an initial inoculum density of 10^5 cells per culture vessel. After 4 days of growth, the monolayers were washed and provided with fresh medium free of serum and containing the sugars indicated at the concentrations given. Twenty-four hours later the rate of uptake of radioactive glucose ([U-^{14}C]glucose) was determined on triplicate culture vessels for each. Uptake is expressed as picomoles per milligram of cell protein accumulated during a 5-min assay.

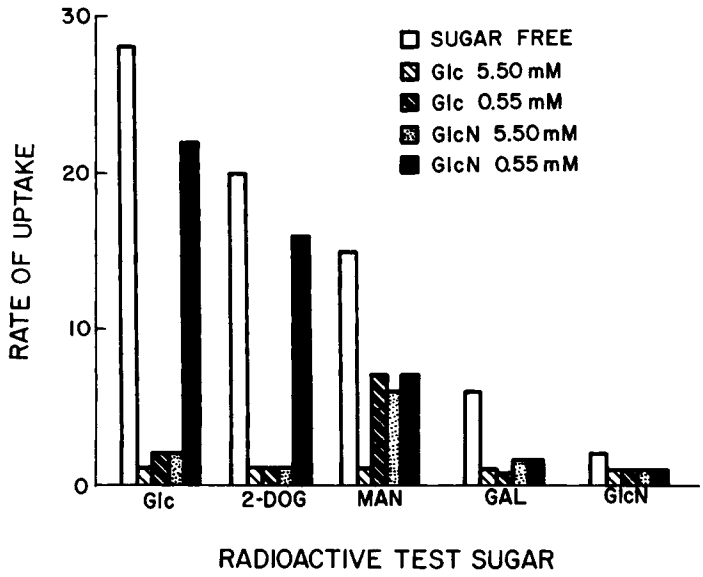

Fig. 2. Asymmetry of derepression of transport of related hexoses. Cells prepared as described for Fig. 1. Twenty-four hours of conditioning was accomplished without glucose or with glucose or glucosamine at concentrations indicated on the bars. The rate of uptake of each of the 5 radioactive hexoses was determined on triplicate cultures. Uptake is expressed as picomoles of hexose accumulated per milligram of cell protein during a 5-min assay. The values were normalized with the rate of uptake for each hexose by cells fed glucose at 5.5 mM concentration arbitrarily set as 1. [Reprinted from Musliner et al (13), with permission.]

elements are responsible for the transport of the several sugars, the complex of transport and phosphorylation is subject to subtle differences suggesting that common and unique elements are involved.

High- and Low-Affinity Carriers

Cells derepressed for glucose transport by starvation proved to possess a second carrier for glucose. The K_m of the derepressible carrier (12) proved to be about 20-fold lower (K_m = 40–50 μM) than that of the constitutive carrier (K_m = 1 mM) (Table I). The high-affinity carrier is inhibited by N-ethylmaleimide (12) while the constitutive carrier is unaffected by that reagent. Moreover, the high-affinity carrier has a much lower relative affinity for 2DOG and 3-O-methyl glucose than the constitutive carrier that favors 2DOG. In addition to derepression of a high-affinity carrier, there is also evidence for derepression of the low-affinity carrier in the change in V_{max} demonstrated for that carrier (Table I) (12).

Derepression Blocked by Cycloheximide and Actinomycin D

Both protein and RNA synthesis appear to be required for derepression initiated by starvation (1, 7, 12, 13). Cycloheximide (Fig. 3) added at any point to cells deprived of glucose blocks the further increase in transport rate. Puromycin, actinomycin D, and cordycepin (13) have a similar effect. As reported in previous publications (13, 17, 20) insulin further increases the transport rate when it is used to treat cells deprived of sugar.

TABLE I. Properties of Glucose Carriers in Chick Fibroblasts*

Conditioning (18–24 h)	Sugar transported	Carrier	K_m (mM)	V_{max} (nmol/min/mg)
Glc (5.5 mM)	D-Glucose	I	1.0	2–3
	2-deoxy-Glc		1–2	6–7
	3-O-methyl Glc		2–3	3–4
	D-Glucose	II	0.04–0.05	0.4
	2-deoxy-Glc		–	–
	3-O-methyl Glc		–	–
Sugar free	D-Glucose	I	1.0	10
	2-deoxy-Glc		1–2	15–20
	3-O-methyl Glc		3–4	20–25
	D-Glucose	II	0.04–0.05	4–5
	2-deoxy-Glc		–	–
	3-O-methyl glc		–	–

*CEF cultures were grown on BME for 3–4 days and, after washing, reincubated in medium containing dialyzed serum and either no added sugar or glucose (5.5 mM) for 18–24 h. The transport assays were initial rates from 10 second assay periods (12). The concentrations of sugars ranged between 5 μM and 1.25 mM for each sugar. The computations of K_m and V_{max} were as explained in the earlier report of Christopher, Kohlbacher, and Amos (12).

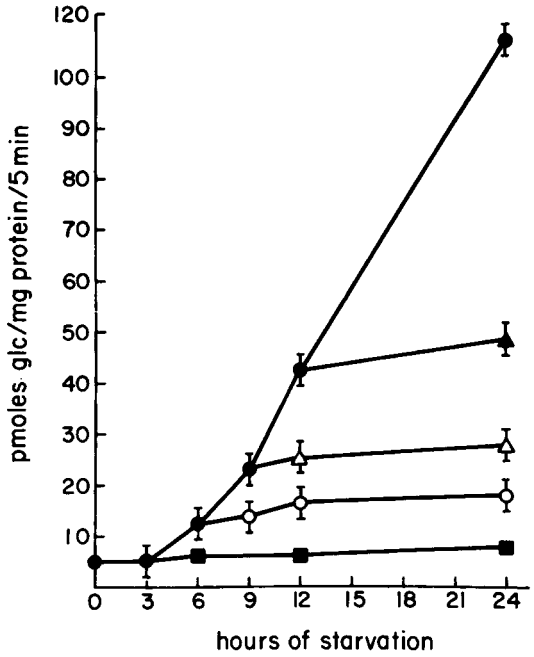

Fig. 3. Cycloheximide inhibition of derepression.,Conditions as in Fig. 1. •) No cycloheximide; ▪) cycloheximide (10 μg/ml) at 0 time; ○) cycloheximide at 6 h; △) cycloheximide at 9 h; ▲) cycloheximide at 12 h.

The stimulation attributed to insulin, like that induced by starvation, is blocked by inhibitors either of RNA or protein synthesis (Fig. 4).

Evidence for Putative Carrier mRNA

If indeed the removal of glucose from the culture medium sets in motion the synthesis of a new carrier or increases the rate of synthesis of an existing carrier, the accumulation of mRNA for one or both carriers should be demonstrable. Many efforts to achieve continued increase in transport after addition of actinomycin to starved cultures failed. Since the interruption of putative mRNA synthesis should not theoretically abruptly terminate translation, we sought other explanations for the blockage by actinomycin D. Indeed we found that if derepression is effected by the replacement of glucose with D-xylose (6.6 mM) or D-fructose (2.75 mM), actinomycin D is not inhibitory except in the starved cells (Fig. 5). D-Xylose and D-fructose were chosen because they have been shown to be "non-repressive" sugars (1, 18) for chick cell glucose transport at the concentrations employed here (Fig. 1). This we took as presumptive evidence for the involvement of energy depletion in the actinomycin D inhibition. Interference with glucose metabolism has been reported as a side effect of actinomycin D treatment of some mammalian cells (25).

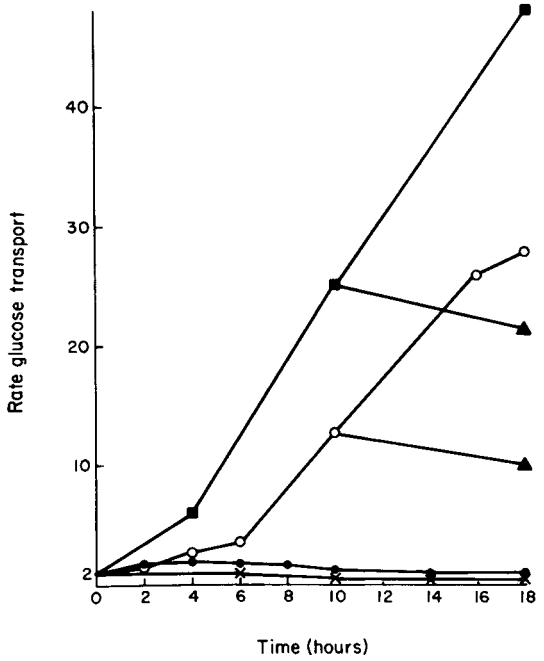

Fig. 4. Inhibition of derepression by actinomycin D and cycloheximide. Cells were grown for 4 days as described for Fig. 1. The monolayers were washed and provided with fresh medium with the sugars indicated or sugar-free. Some monolayers received insulin (10^{-1} U/ml) without sugar. At 10 h actinomycin D (2 μg/ml) or cycloheximide (10 μg/ml) was added to some insulin-stimulated as well as to some starved cultures. As in Fig. 2 the rate of glucose uptake in cells provided with glucose has been arbitrarily set as 1. ○) Starved cultures; ■) insulin (10^{-1} U/ml; ×) cycloheximide (10 μg/ml) alone; ●) glucose (5.5 mM).

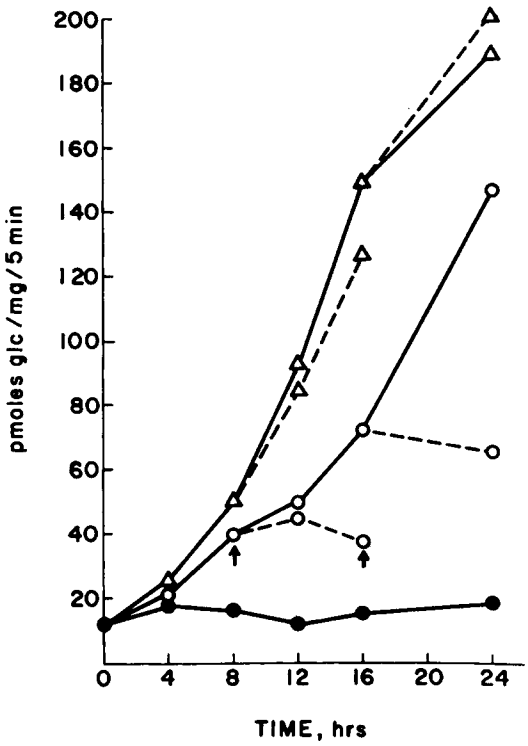

Fig. 5. Refractoriness of derepression to inhibition by actinomycin D. Chick embryo fibroblast (CEF) cells were grown for 3 days on BME, glucose (5.5 mM), and 4% calf serum. At that time all cultures were washed then provided with fresh serum-free BME containing: △—△) D-xylose (6.6 mM); •—•) D-glucose (5.5 mM); ○—○) no sugar. Glucose uptake was measured at intervals (5-min assay) with triplicate cultures per time point. Actinomycin D (5 μg/ml) was introduced at the 8th and 16th hours (indicated by arrows): △- - -△) D-xylose BME; ○- - -○) sugar-free BME.

Evidence for increased potential to derepression was obtained by employing cycloheximide to inhibit carrier synthesis while permitting mRNA to accumulate as has been shown to occur in enzyme induction in chick cells by hydrocortisone (14, 15). Thus cells deprived of glucose and simultaneously inhibited with cycloheximide (10 μg/ml) for 20 h exhibited a rapid derepression of glucose uptake during the 4–6 h following removal of cycloheximide (Fig. 6). The increase in glucose uptake was observed only when pyruvate (data not shown), xylose, or fructose was provided in the incubation medium, ostensibly as an energy source. The failure to provide an exogenous sugar blocked derepression as did the addition of glucose as the energy source.

Actinomycin D at concentrations ranging from 1 to 5 μg/ml did not inhibit the derepression observed after cycloheximide removal (Fig. 7).

Post-Cycloheximide Derepression of Cells Maintained on Glucose

Whereas cells starved of glucose while inhibited by cycloheximide for 20 h responded to the removal of cycloheximide by rapidly increasing their rate of glucose uptake (Figs. 5–7), cells maintained on glucose without inhibition of protein synthesis showed little

response to removal of glucose over a 6-h period whether or not a nonrepressive energy source was provided (Fig. 8).

If on the other hand glucose (5.5 mM) and cycloheximide were both supplied for 20 h, the removal of both resulted in derepression with a delay of about 2 h (Fig. 8). The extent of derepression at 6 h was considerably greater than that achieved by cells treated with cycloheximide alone (Figs. 6, 7) because the combination of cycloheximide and glucose reduces uptake to a very low level after 20 h (8, 9). The absolute rate of entry at 6 h was not significantly different in the 2 sets. It is also to be noted that the addition of actinomycin D did not alter the derepression of glucose-cycloheximide treated cells, evidence for mRNA accumulation during the 20-h period of inhibition (Fig. 8). Apparently inhibition of protein synthesis by cycloheximide or some other metabolic consequence of cycloheximide treatment results in the availability of mRNA for carrier synthesis whether or not glucose is provided during the 20-h period. Once again the addition of glucose to the post-cycloheximide medium blocked the increase in rate of entry (Fig. 8). In contrast to cells starved during cycloheximide treatment, substantial derepression was achieved without the addition of an energy source to the post-cycloheximide incubation medium (data not shown). Evidently sufficient energy reserves were available in cells provided with

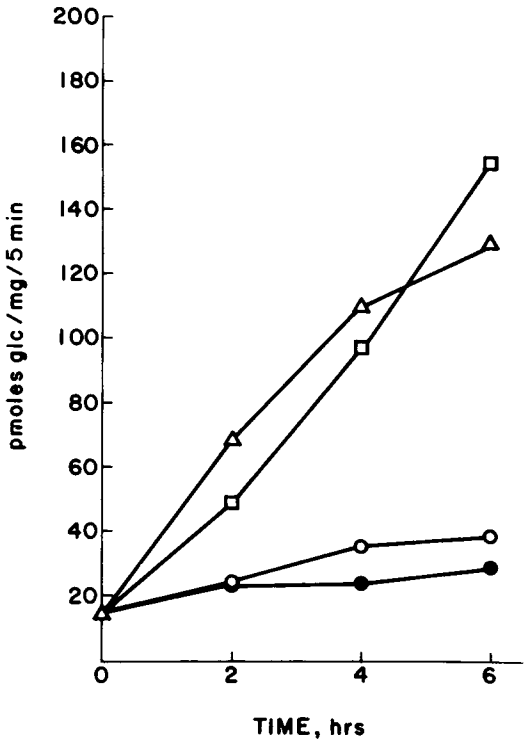

Fig. 6. Derepression after 20-h inhibition by cycloheximide. For all sets except one, cells were conditioned for 20 h with cycloheximide (10 μg/ml) without glucose. At 20 h all cultures were washed 3 times to remove the cycloheximide and were provided with fresh medium with sugars as indicated below: △) D-xylose (6.6 mM); □) D-fructose (5.5 mM); ○) no sugar; ●) D-glucose (5.5 mM).

glucose during the preincubation to permit synthesis of carriers over a 6-h period. The 2-h lag was reproduced in each of the experiments conducted and may well reflect residual "corepressor" formed in the metabolism of glucose.

Evidence for Repression of Glucose Carrier Formation

Let us assume that the blocking of derepression by reagents that inhibit protein synthesis (cycloheximide, puromycin) is in fact due to the inhibition of the formation of new glucose carrier molecules, themselves proteins. When protein synthesis is inhibited the capacity of the cells to transport glucose falls with time such that it is lower at the end of 20 h than the level observed when glucose alone is provided. Christopher and Kalckar have attributed this reduction to the failure to synthesize new carrier molecules coupled with the normal turnover of existing carrier molecules (8, 9). Since, as they have pointed out, the rate of carrier loss is much greater when glucose is present during inhibition by cycloheximide, it is not unreasonable to propose that glucose or a metabolic product of glucose has a role in the rate of carrier turnover. This inference gains support from the failure of glucose to allow the mRNA that accumulates after 20 h of inhibition of protein synthesis to be expressed as new carrier protein. The glucose or a product of glucose alone or in conjunction with a regulatory protein acts: 1) to destroy the carrier mRNA, 2) to block its translation, 3) to inactivate the carrier molecules as they are formed on the polyribosomes, or 4) to destroy formed carrier in transit or after its insertion into the plasma membrane.

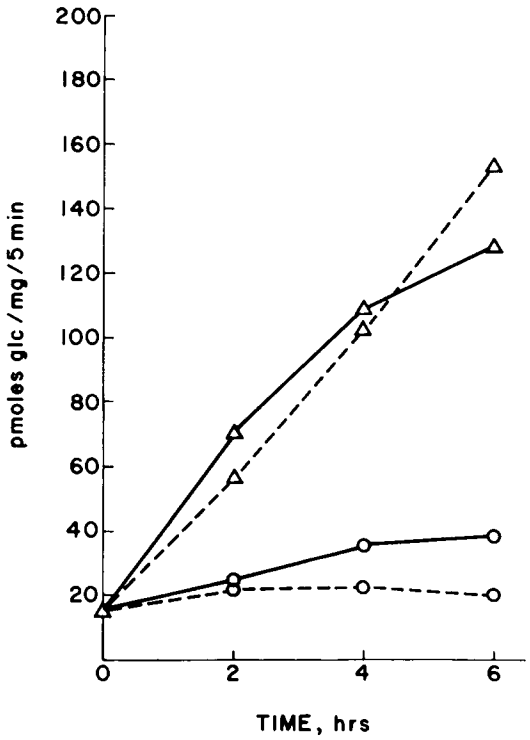

Fig. 7. Actinomycin and post-cycloheximide derepression. Conditions identical to those described for Fig. 6. △—△) D-Xylose (6.6 mM); △ - - - △) D-xylose + Act D (5 μg/ml); ○—○) no sugar; ○ - - - ○) Act D (5 μg/ml).

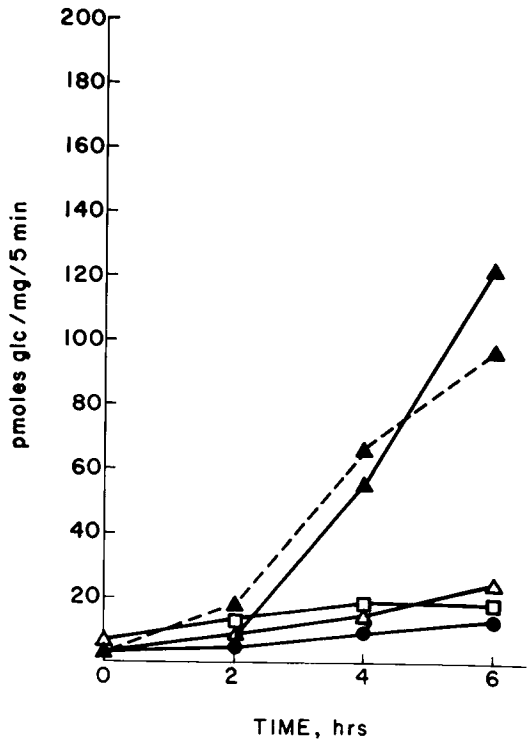

Fig. 8. Derepression after glucose-cycloheximide treatment. Cells were first incubated either with glucose (5.5 mM) alone or with glucose and cycloheximide (10 μg/ml) for 20 h. The post-cycloheximide incubation medium contained sugars as indicated below: △—△) glucose alone followed by D-xylose (6.6 mM) after washing; □—□) glucose alone followed by D-fructose (5.5 mM); ▲—▲) glucose + cycloheximide (10 μg/ml) followed by D-xylose (6.6 mM); ▲- - -▲) glucose + cycloheximide followed by D-xylose (6.6 mM) + actinomycin D (5 μg/ml); ●) glucose + cycloheximide followed by glucose (5.5 mM).

A Model to Account for a Two-Component "Inactivator" of Carrier Protein

The evidence presented above can be reconciled by a model assuming that carrier synthesis is blocked at a posttranscriptional stage and/or that formed carrier is inactivated by a protein (Protein A, Fig. 9) in concert with a cofactor product of glucose metabolism. The protein in question is impotent except when modified by the cofactor to become the "inactivator." The protein can be considered to have a finite half-life, perhaps a short half-life, so that it is reduced significantly in concentration when protein synthesis is arrested. Thus after an extended period of inhibition of protein synthesis little Protein A exists, though its mRNA may well have accumulated.

Glucose-Induced Decay of Transport

Evidence lending support to the "Protein A-Cofactor-Inactivator" model has been presented by Christopher and Kalckar (8, 9) in experiments in which they demonstrate the reversal of derepression in Nil cells by addition of glucose to highly derepressed cells. Carrier function is sharply reduced with a half-decay time estimated at approximately 2.5 h. Cycloheximide protects the carriers from loss if it is added before or simultaneously with glucose.

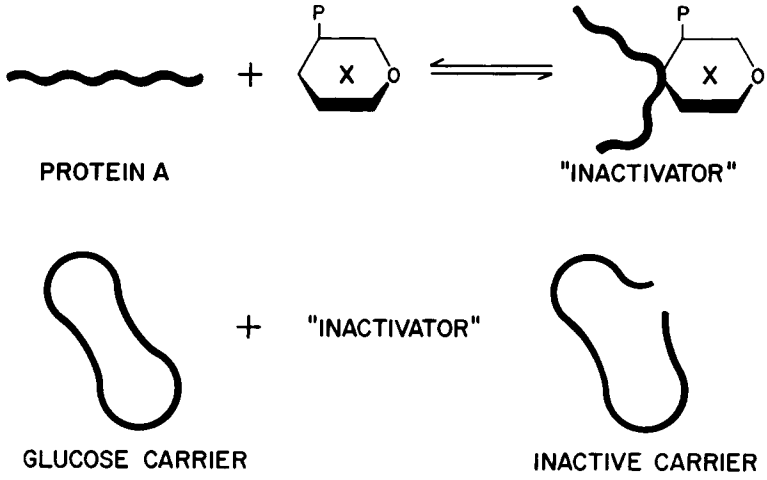

Fig. 9. Two-component "Inactivator" of glucose carrier.

We have been able to confirm the essential findings of Christopher and Kalckar using chick cells (13) (Fig. 9). The rate of decay of transport function is dependent upon the concentration of glucose, as is the protective effect of cycloheximide. Glucose at a concentration of 5.5 mM reduces uptake with a half-decay time of less than 15 min. A 10-fold reduction in the concentration of glucose extends the half-decay time to approximately 3 h. The decay of transport effected by glucose at either concentration is not reversed by removal of the sugar or its replacement by D-xylose or D-fructose (data not shown). Cycloheximide added before glucose or simultaneously with it reduces the rate of loss of carrier function (Figs. 10 and 11). Nearly full protection is achieved when the glucose concentration is lowered to 0.55 mM. Actinomycin D, on the other hand, is unable to block the loss of carrier function even with the lowered glucose concentration (Fig. 11).

We interpret the loss of carrier function to represent the inactivation of carrier already inserted into the plasma membrane. During starvation much less Protein A is formed, more in chick fibroblasts than in Nil cells. The addition of glucose permits an early burst of Protein A synthesis from accumulated mRNA. Cycloheximide blocks the formation of Protein A and the system for carrier inactivation is not operative despite a high level of the glucose cofactor. Actinomycin D does not have a protective effect because mRNA for Protein A synthesis is available and is translated as soon as glucose is supplied. 2-Deoxy-D-glucose (8) does not mimic glucose in initiating carrier destruction in Nil cells. With chick fibroblasts the cells are further derepressed when 2DOG is supplied in lieu of glucose. There is as yet no clear explanation of the 2DOG effect. Among the most interesting aspects of the putative carrier inactivating system is the conjecture that it is a model for assigning specificity to protease-mediated protein degradation in mammalian cells. Inhibitors of protease action may prove helpful in further study of glucose carrier decay.

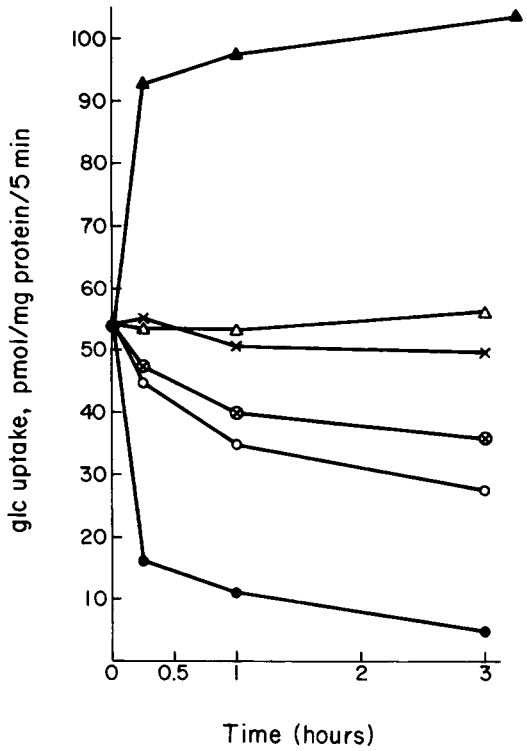

Fig. 10. Transport decay in derepressed cells. Cells were starved for 24 h, washed, and provided with fresh medium with the components indicated. At intervals after initiation of the experiment triplicate monolayers were assayed for glucose uptake with [U-^{14}C]glucose (5-min assay). •) glucose (5.5 mM); ○) glucose (0.55 mM); ▲) DOG (0.55 mM); △) cycloheximide (10 μg/ml); ⊗) glucose (5.5 mM) + cycloheximide (10 μg/ml); ×) glucose (0.55 mM) + cycloheximide (10 μg/ml).

DISCUSSION

The results presented here are considered presumptive evidence for a regulatory mechanism operating to control the density of glucose carriers in the chick fibroblast membrane. It is now well established that the uptake measured in chick fibroblasts is indeed a measure of transport and not of rate-limiting phosphorylation (7, 8, 9, 12, 13). In fact the derepression resulting from removal of glucose from the cell culture medium or its replacement by "nonrepressive" sugars such as xylose and fructose (1, 13, 18) has also been shown by the use of 3-O-methyl glucose to represent an increase in transport rather than of coupled metabolism of glucose or 2-deoxy-D-glucose (7, 12, 13). Transport appears to be limiting both in the repressed and derepressed chick fibroblast.

Christopher et al. (12) concluded that starvation for glucose resulted in the derepression of 2 carrier systems for glucose in the chick cell. One, a constitutive carrier (K_m = 1 mM) and a second higher affinity carrier (K_m = 0.04–0.05 mM) were derepressed, the second severalfold in the absence of glucose. Banjo and Perdue (26) identified 2 membrane

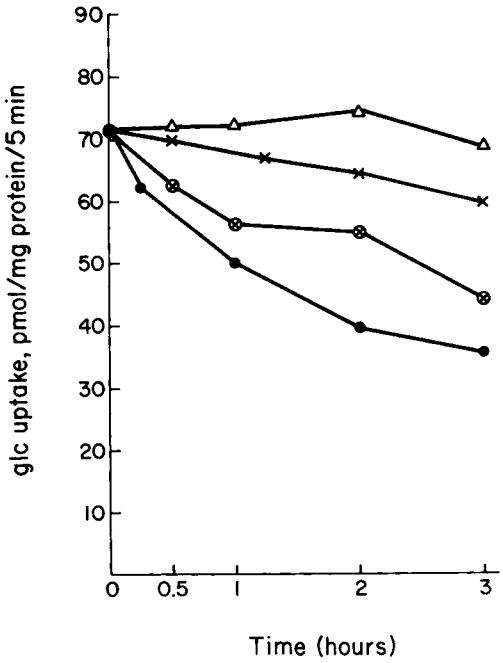

Fig. 11. Actinomycin versus cycloheximide as antagonist of transport decay. Conditions same as for Fig. 10. Test additions as indicated below: •) glucose (0.55 mM); X) glucose (0.55 mM) + cycloheximide (10 μg/ml); ⊗) glucose (0.55 mM) + Act D (5 μg/ml); △) cycloheximide (10 μg/ml).

proteins (approximate molecular weights 95,000 and 75,000) whose rate of synthesis was accelerated when chick cells were starved of glucose. Pastan and collaborators have confirmed and extended (27) the observations of Banjo and Perdue. The implication of the specific repression of both proteins that results from maintaining high glucose concentrations in the culture medium is that both proteins may be involved in glucose transport.

The previously reported inhibition of derepression by actinomycin D (7, 13) is herein shown to be attributable in all probability to an energy deficit. Supplying a "nonrepressive" source of energy such as xylose, fructose, or pyruvate makes derepression of glucose transport refractory to actinomycin D while retaining the sensitivity to inhibitors of protein synthesis.

By inhibiting glucose carrier formation with cycloheximide for an extended period, rapid derepression has been shown to occur upon removal of cycloheximide. The introduction of actinomycin D to block further RNA synthesis does not inhibit the rapid increase in transport during a 6-h period. The derepression achieved in 6 h virtually equals that observed only after 24 h of starvation. Again a "nonrepressive" source of energy is required for full expression. Whether or not glucose is present during the long period of interruption, the release from cycloheximide results in a rapid increase in transport function.

Significantly, glucose supplied as energy source with or without actinomycin blocks derepression on removal of cycloheximide. The tentative interpretation is that glucose exerts a posttranscriptional effect: 1) by destruction of preformed mRNA, 2) by inhibiting translation of mRNA, or 3) by a mechanism that results in inactivation of the carrier

as it is being formed or after its insertion into the membrane. Acceleration of the inactivation of carrier already resident in the membrane is observed when glucose is added to derepressed cells (8, 9, 13). The rate of transport reduction is a function of glucose concentration. 2-Deoxyglucose is not active in accelerating the inactivation of carrier. It in fact promotes a paradoxical enhancement of transport.

Thus the role of glucose or a close metabolic derivative of glucose in the regulation of glucose carrier density has 2 potential facets: 1) that of posttranscriptional corepressor and 2) that of signal for carrier inactivation.

Derepression of glucose transport in Neurospora crassa (28, 29) parallels that observed with chick and mammalian cells. In Neurospora a second carrier of lower K_m and capable of active transport is derepressed when cells are starved of glucose or provided with fructose in lieu of glucose for several hours. The inactivation of the carriers is likewise triggered by the addition of glucose to the incubation medium and is concentration dependent. Evidence has been presented implicating protease action in the inactivation of the transport system with kinetics that resemble those observed with chick cells.

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