Regulation of Hexose Carriers in Chicken Embryo Fibroblasts

EFFECT OF Glucose Starvation AND ROLE OF PROTEIN SYNTHESIS*

Kazu Yamada, Loyal G. Tillotson, and Kurt J. Isselbacher

From the Department of Medicine, Harvard Medical School and Massachusetts General Hospital (Gastrointestinal Unit), Boston, Massachusetts 02114

Regulation of hexose transport was investigated in chicken embryo fibroblasts (CEF) which develop 4- to 8-fold enhanced hexose transport activity during glucose starvation. The presence of cycloheximide in low (0.5 μg/ml) concentrations during starvation largely blocked the enhancement of transport activity. Glucose refeeding of CEF in the starvation state led to a decline in transport in the basal level. This decline was either potentiated or blocked by the presence of cycloheximide in low or high (50 μg/ml) concentrations, respectively. Exposure of CEF in the fed state to low concentrations of cycloheximide resulted in a 70% decrease of transport within 6 h, whereas exposure to high concentrations of cycloheximide led to only a modest loss (35% decrease). In the glucose-starved state, CEF had no significant decline of transport when exposed to cycloheximide at either high or low concentrations. The uptake of 3-O-methylglucose by fed, starved, or cycloheximide-treated CEF correlated closely with D-glucose transport activity and [3H]cytochalasin B binding by plasma membranes prepared from CEF exposed to the same conditions. Hexose transport activity of CEF seems to largely depend on the number of functioning carriers in the plasma membrane, which apparently reflect the balance between carrier synthesis and inactivation. These two processes require protein synthesis, but are differentially sensitive to the effects of cycloheximide, such that low concentrations of cycloheximide appear to block primarily synthesis while high concentrations block both processes. Furthermore, during starvation the enhancement of transport appears largely due to decreased carrier inactivation in the face of continued carrier synthesis.

Cultured animal cells when deprived of D-glucose in their growth medium manifest a striking adaptive response by developing greatly enhanced hexose uptake ability (1-10). This response can be elicited or maintained by substituting fructose, xylose, 2-deoxyglucose, or 3-O-methylglucose for D-glucose in the growth medium (4, 5, 9, 10) or under starvation or feeding of CEF. The enhancement of transport from glucose starvation can be largely blocked by treatment with inhibitors of protein or RNA synthesis (1, 2, 9, 12). In addition, the presence of these inhibitors can prevent the decline of transport activity that normally occurs when starved cells are re-fed with glucose-containing culture medium (4, 12, 13). Since the starvation effects seem to depend in part on protein synthesis, Christopher et al. have proposed that regulation of hexose carriers results from ongoing processes of carrier synthesis and inactivation, both of which are dependent on protein synthesis (13).

The increase of carriers in the plasma membrane during glucose starvation takes several hours to develop and requires protein synthesis. This indicates that new carrier synthesis probably accounts for most of the increase rather than translation of an intracellular pool of preformed hexose carriers. Details of the mechanism for inactivation of hexose carriers are not yet known and could comprise several steps including structural modification or internalization and subsequent degradation. For the sake of simplifying the discussion we have used the term "carrier inactivation" to describe this unknown mechanism. Recent work by Kalckar and his colleagues suggested that carrier inactivation relies on oxidative energy metabolism by both the pyruvate-tricarboxylate cycle and glucose-6-phosphate pentose shunt (15, 16). In addition, Christopher and Morgan have evidence that lysosomal cathepsin activity is involved as well (17).

In the current study we have attempted to elucidate the role of protein synthesis in hexose transport regulation of chicken embryo fibroblasts. We investigated the effects of cycloheximide on modulation of transport activity during starvation or feeding of CEF. Transport activity was correlated with the relative number of functioning carriers in plasma membranes derived from the cells. These results have led us to postulate possible mechanisms for the regulation of synthesis and inactivation of hexose carriers. Furthermore, these findings offer new support for Christopher's hypothesis of glucose carrier turnover, based on similar findings with hamster cells (13, 29).

METHODS

Cell Culture—Chick embryo fibroblasts were prepared from 10-day-old embryos by modification of methods previously described (18). Briefly, body walls from chick embryos were dissociated with 0.25% trypsin and cells were seeded into plastic roller bottles (Corning 850 cm2) containing 150 ml of Dulbecco's modified Eagle's medium containing 4.5 g/liter of glucose, 10% tryptose phosphate broth, and 2% irradiated fetal calf serum. After 2-5 days as primary cultures at 41 °C, the cells were trypsinized with 0.06% trypsin, 0.5 mM EDTA in phosphate-buffered saline without Mg2+ and Ca2+, and transferred into fresh medium containing 4.5 g/liter of glucose and 10% calf serum.

* This work was supported by United States Public Health Service Grants AM01292, AM03014, and AM07191. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 In this manuscript the term "carrier" is used synonymously with the "facilitated hexose transporter" of plasma membranes.

2 The abbreviations used are: CEF, chicken embryo fibroblasts; DPBS, Dulbecco's-buffered saline.
at 2 × 10^6 cells/plate into 35-mm plastic dishes (Lux). The secondary cultures were incubated at 41°C in a humidified CO₂ incubator with 10% CO₂ for 2 days. In membrane preparation experiments, the secondary cultures were seeded at 2–3 × 10^6 cells in roller bottles and incubated at 41°C for 3 days to reach confluency. CEF in 35-mm dishes and roller bottles received fresh culture medium 20–24 h before assaying transport or harvesting cells for membrane preparation. The cultures were dissolved of Dulbecco’s modified Eagle’s medium without glucose and supplemented with 2% dialyzed fetal calf serum, 10% glucose-free tryptose phosphate broth, and either D-glucose or fructose (final concentration 25 mM).

**Preparation of Membrane Vesicles**—Mixed membrane vesicles were prepared by a nitrogen cavitation method previously described (18). Membranes were washed once in 0.1 M sorbitol and 1 mM Tris-Cl (pH 7.5) (buffer S), and suspended to give a final protein concentration of 3–4 mg/mL by passage through a fine-gauge needle.

**Transport Assay of Whole Cells**—After aspirating culture medium, each dish was washed twice with 2 mL of prewarmed (37°C) sugar-free Dulbecco’s buffered saline. The cells which remained anchored to the dish were preincubated in sugar-free DPBS at 37°C for 5 min. The DPBS was replaced with prewarmed DPBS containing 0.1 mM 3-O-[[14C]methyl]glucose (1 μCi/mL) and 0.1 mM L-[3H]glucose (2 μCi/mL; New England Nuclear) for 10 s. The transport rates were followed at 3°C in glucose-starved CEF (which showed the highest rate). Following aspiration of isotope solution, cells were quickly washed with 10 mL of ice-cold DPBS with 0.1 mM phloretin. Then 1 mL of 0.1 M NaOH, 0.1% sodium deoxycholate, and sucrose solution was added to the supernatant and aliquots were taken for protein determination and liquid scintillation counting. Corrections for simple diffusion and nonspecific absorption were corrected by subtracting the amount of L-[3H]glucose associated with each sample. Transport assay was also measured with 2-deoxyglucose which in all cases confirmed the results of 3-O-methylglucose uptake (data not shown).

**Measurement of Leucine Incorporation**—The degree of leucine incorporation into acid-insoluble material was measured by the method described by Wardzala et al. (20). The membrane suspension was mixed with cytochalasin B and buffer S containing either D-sorbitol or D-glucose. Aliquots (100 μl) from each were transferred to polycarbonate centrifuge tubes (Beckman) and mixed with 20 μl of 0.9 solution of 240 mM [3H]cytochalasin B (0.033 μCi/μl; New England Nuclear) and [14C]sucrose (0.017 μCi/μl) in buffer S containing 6 mM CaCl₂, MgCl₂. The final concentration of ethanol, cytochalasin B, and D-sorbitol were, respectively, less than 1%, 2 μM, 500 mM, and 500 μM. The samples were incubated for 10 min at room temperature and then centrifuged at 150,000 × g for 30 min. Pellets in the tubes were solubilized with 300 μl of 0.1 M NaOH, 0.1% sodium deoxycholate, and an aliquot taken for counting.

**Cytochalasin B Binding**—Cytochalasin B binding was measured by the method described by Wardzala et al. (20). The membrane suspension was mixed with cytochalasin B and buffer S containing either D-sorbitol or D-glucose. Aliquots (100 μl) from each were transferred to polycarbonate centrifuge tubes (Beckman) and mixed with 20 μl of a solution of 240 μM [3H]cytochalasin B (0.033 μCi/μl; New England Nuclear) and [14C]sucrose (0.017 μCi/μl) in buffer S containing 6 mM CaCl₂, MgCl₂. The final concentration of ethanol, cytochalasin B, and D-sorbitol were, respectively, less than 1%, 2 μM, 500 mM, and 500 μM. The samples were incubated for 10 min at room temperature and then centrifuged at 150,000 × g for 30 min. Pellets in the tubes were solubilized with 300 μl of 0.1 M NaOH, 0.1% sodium deoxycholate, and an aliquot taken for counting.

**Data Calculations and Reproducibility**—Data presented were from individual experiments which, usually, were repeated three times. Each transport data point was the mean value of three samples. Standard error in most cases was ±2%.

### Results

**Effect of Cycloheximide on Hexose Transport Activity**—The role of protein synthesis in the mechanisms of hexose transport regulation in CEF was investigated with cycloheximide, a potent inhibitor of protein synthesis. Hexose transport activity was determined by assaying the uptake of 3-O-methylglucose, a nonmetabolizable substrate of the glucose transport system. The effects of cycloheximide differed depending on the concentration used and whether CEF were in the glucose fed state or in a starvation state. As shown in Fig. 1A, when CEF were in a fed state treated with low concentrations of cycloheximide (0.5 μg/mL), hexose transport activity declined rapidly with only a short lag period; by 8 h, transport was approximately 20% of the control activity (t₁/₂ = 3 h). Treatment of fed CEF with high concentrations of cycloheximide (50 μg/mL) resulted in gradual reduction of transport activity but only to a level of about 65% of control at 8 h (t₁/₂ = 15 h). As previously demonstrated by others (1, 2, 9, 10), hexose transport activity of starved CEF was approximately 6-fold greater than fed CEF (Fig. 1B). This enhanced transport activity of starved CEF was largely unaffected by treatment with either high or low concentrations of cycloheximide. Glucose refeeding of starved CEF (S → F) resulted in a decline of 3-O-methylglucose transport activity to the usual fed CEF level (i.e., from about 300 to approximately 60–90 pmol/mg of protein/10 s) (t₁/₂ = 3.5 h). When low concentrations of cycloheximide were present during glucose refeeding of starved CEF the decrease in transport activity was accelerated (t₁/₂ = 2.5 h) and somewhat greater (at 8 h) than with refeeding alone. However, glucose refeeding with high concentrations of cycloheximide resulted in only a minimal decrease in transport from the starved state. The above observations were confirmed by determining efflux of substrate from fed, starved, or cycloheximide-treated CEF. Since the facilitated hexose carrier functions bidirectionally, cells which have the highest hexose uptake activity also have the greatest efflux activity. The efflux experiments using CEF preloaded with 3-O-[[14C]methylglucose yielded the same pattern of results as the influx experiments (data not shown).

**Concentration-Dependent Effects of Cycloheximide on Hexose Transport Activity**—Cycloheximide has been shown to prevent the enhancement of transport which normally occurs with starvation of CEF (1, 2, 9). This phenomenon was further examined by studying the effect of various cycloheximide concentrations on the transport activity of fed CEF (pretreatment condition) which were then either maintained in the fed state or were starved for a treatment period of 6 h. Hexose transport activity was determined at the end of the treatment period. As shown in Fig. 2A fed CEF experienced a greater loss of transport with low concentrations of cycloheximide (0.5–1 μg/ml) than with high concentrations of cycloheximide (50 μg/mL). This dose response effect is in good agreement with a previous report of cycloheximide treatment of fed hamster cells by Christopher et al. (13). The effects of cycloheximide on CEF undergoing starvation are also shown in Fig. 2A. Compared with the enhanced transport of CEF starved for 6 h in the absence of cycloheximide (control), increasing concentrations of cycloheximide blunted, although

---

3To note the changes in incubation conditions we have used the following abbreviations: CX-H = high concentration cycloheximide (50 μg/ml); CX-L = low concentration cycloheximide (0.5 μg/ml); S = starvation (fructose-containing medium); F = fed (glucose-containing medium); AMD-H = high concentrations actinomycin D (10 μg/ml). Thus, for example S → F + CX-L = starved cells switched to glucose refeeding in presence of low concentration cycloheximide; S → S means cells received a medium change but were maintained in the starved state.
Fig. 1. Cycloheximide treatment of CEF. Cultured CEF were pretreated for 20-24 h in the fed (A) or starved state (B). Fed medium contained 25 mM D-glucose [F]; starvation medium contained 25 mM fructose [S]. The experiments were initiated at culture time = 0 by a change of fresh medium with or without cycloheximide (Cx-L, 0.5 μg/ml of cycloheximide; Cx-H, 50 μg/ml of cycloheximide). Uptake of 3-O-methylglucose (3-OMG) was determined at the indicated times as described under "Methods." Each data point represents the mean value of three samples.

Fig. 2. Concentration effect of cycloheximide treatment of CEF. Cultured CEF were pretreated for 20-24 h in the fed (A) or starved state (B). Fed CEF then received a medium change to continued fed or starvation [F → S] conditions with or without cycloheximide (0.1-50 μg/ml). Starved CEF received a similar medium change to continued starvation or to fed [S → F] conditions. After 6 h of treatment, 3-O-methylglucose (3-OMG) uptake was determined. Each value was the mean of three samples. The relative incorporation of [3H]leucine into acid-insoluble fractions was determined as described under "Methods."
brane vesicles are presented in Table I. D-Glucose-inhibitable hexose carrier sites (20, 22-25). Plasma membrane vesicles of CEF and other animal cells. The amount of cytochalasin B binding that is inhibitable by D-glucose has been shown to be approximately 10 mM for all membrane preparations. Membrane vesicles from refed cells treated with low concentrations of cycloheximide have a relatively low hexose carrier inactivation rate.

Kinetics of D-Glucose Transport by Membrane Vesicles—Analysis of transport mechanisms of whole cells can be complicated by factors such as substrate metabolism, intracellular compartmentalization, and substrate pool size. However, by utilizing plasma membrane fractions, membrane transport properties can be separated from intracellular events. Inui et al. (18) and Zala and Perdue (21) have shown that plasma membrane vesicles isolated from CEF retain hexose transport properties of intact cells. Studies were therefore carried out on membrane vesicles derived from cells cultured under conditions similar to those used in Fig. 1B. Plasma membrane vesicles were prepared from cells initially starved 20 h and then prior to harvesting were treated for 6 h by further starvation [S → S] or glucose refeeding, with or without cycloheximide [S → F, F + Cx-H or Cx-L]. Transport kinetics were determined by measuring stereospecific glucose uptake over a d-glucose concentration range of 0.5-10 mM with an uptake time of 5 s. The kinetic results were graphed as Eadie-Hofstee plots (Fig. 4). The K, of glucose transport was approximately 10 mM for all membrane preparations. Membrane vesicles isolated from starved CEF had the highest V, max, i.e., 6 nmol/mg/5 s, which was 4-fold greater than membrane vesicles from refed cells. Membrane vesicles from refed cells treated with high concentrations of cycloheximide had an intermediate V, max of 3 nmol/mg/5 s, whereas membrane vesicles from refed cells treated with low concentrations of cycloheximide had the lowest V, max of 1 nmol/mg/5 s. These results of the functional carrier activity at the membrane level are consistent with hexose transport activity reported above using cultured cells (Fig. 1B).

D-Glucose-inhibitable Cytochalasin B Binding Activity of Membrane Vesicles—Cytochalasin B is a specific inhibitor that binds with high affinity to the hexose transport system of CEF and other animal cells. The amount of cytochalasin B binding that is inhibitable by D-glucose has been shown to be an indirect but relatively quantitative measure of total hexose carrier sites (20, 22-25). Plasma membrane vesicles were prepared using culture conditions exactly as described above. The results of cytochalasin B binding to plasma membrane vesicles are presented in Table I. D-Glucose-inhibitable binding represents the difference in binding in the presence of 0.5 M sorbitol and 0.5 M D-glucose. Again, findings similar to the membrane vesicle transport kinetics (Fig. 4) and whole cell transport data (Fig. 1B) emerged—namely, membranes from starved CEF had the highest cytochalasin B binding activity. Thus, an excellent correlation was observed between D-glucose-inhibitable binding sites of cytochalasin B and the functional hexose transport changes observed with membrane vesicles.

Delayed Response of Transport Regulation following Cycloheximide Treatment of Starved CEF—The above data indicate that protein synthesis is required for carrier synthesis and inactivation. To further characterize the requirements for carrier inactivation during glucose refeeding of starved cells, the rate of expression of inactivation was examined following a period of complete inhibition of protein synthesis. CEF were

![Fig. 3. Rate of decline of transport activity by refeeding starved CEF. Starved CEF were pretreated for 4 h with serum-deficient starvation medium containing 0.5 μg/ml of cycloheximide. Culture media were changed at 4 h to starvation +0.5 μg/ml of cycloheximide (control), fed +50 μg/ml of cycloheximide (F + Cx-H), or fed (F) serum-deficient media. Uptake of 3-O-methylglucose (3-OMG) was determined at the indicated times. Each data point was the mean value of three samples.](http://www.jbc.org/)

![Fig. 4. Kinetics of D-glucose uptake by CEF plasma membrane vesicles. Starved CEF were treated with starvation [S], fed +50 μg/ml of cycloheximide [F + Cx-H], fed +0.5 μg/ml of cycloheximide [F + Cx-L], or fed [F] serum-deficient media for 6 h. Cells were then harvested and plasma membranes prepared as described under "Methods." Net uptake of D-glucose (D-[¹C]glucose minus L-[¹H] glucose) was determined over a concentration range of 0.5-10 mM glucose and an uptake time of 5 s. Data points are the mean of duplicate determinations and are presented as an Eadie-Hofstee plot. The K, values for all membrane preparations were approximately 10 mM. The V, max values listed in the inset have units of nanomoles/mg of protein/5 s.](http://www.jbc.org/)

### Table I

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>0.5 M sorbitol (A)</th>
<th>0.5 M D-glucose (B)</th>
<th>Glucose inhibitable (A - B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>5.6 ± 0.8</td>
<td>2.2 ± 0.4</td>
<td>3.4</td>
</tr>
<tr>
<td>F + Cx-H</td>
<td>4.3 ± 0.07</td>
<td>2.0 ± 0.02</td>
<td>2.3</td>
</tr>
<tr>
<td>F</td>
<td>3.0 ± 0.10</td>
<td>1.8 ± 0.10</td>
<td>1.2</td>
</tr>
<tr>
<td>F + Cx-L</td>
<td>2.8 ± 0.04</td>
<td>1.8 ± 0.09</td>
<td>1.0</td>
</tr>
</tbody>
</table>
starved 20–24 h prior to the start of the experiment. As shown in Fig. 5 the starved cells had a change of medium at culture time zero, such that some cells received glucose refeeding medium (Fig. 5A) while other cells received starvation medium (Fig. 5B) but both were exposed to high concentrations of cycloheximide. Cells treated with cycloheximide are able to transcribe stable species of mRNA which can subsequently be expressed upon removal of the cycloheximide (26, 27). As expected, during the first 4 h of cycloheximide treatment the transport activity remained at an enhanced level regardless of glucose refeeding (Fig. 5A) or continued starvation (Fig. 5B). After 4 h of treatment, the cells were rinsed with DPBS and had another change of medium—namely, to starvation medium, starvation plus high concentrations of cycloheximide, fed (glucose-containing) medium, or fed plus high concentrations of actinomycin D. The response of the cells now differed depending on whether the cells had been starved or refeed with glucose in the previous 4 h. Thus, as shown in Fig. 5A, cells fed during cycloheximide treatment, had a precipitous drop in transport activity when exposed to fed or fed plus actinomycin D media. Cells which received starvation medium experienced a rapid but partial drop in transport activity. However, cells kept in the presence of cycloheximide maintained the elevated transport activity. This suggests that, following the 4 h of glucose refeeding plus high concentration cycloheximide, the cells receiving medium without cycloheximide were immediately able to renew carrier inactivation because either the mRNA necessary for that process had accumulated or the existing mRNA was more available for expression in translation.

As shown in Fig. 5B, cells which had initially been treated with starvation plus cycloheximide reacted much differently to the change of medium at 4 h. Although the cells refeed with glucose lost transport activity to the same basal level as the refeed cells in Fig. 5A, the rate of decline was not as rapid. The cells refeed with glucose plus actinomycin D had only a slight loss of transport activity indicating that once the cycloheximide block was released, the refeeding effect was prevented by the presence of high actinomycin D concentrations. The same phenomenon was observed in cells changed to starvation medium without cycloheximide. These results are in sharp contrast to those shown in Fig. 5A and suggest that during the period of starvation plus cycloheximide there was either no accumulation of mRNA for carrier inactivation, or any existing mRNA for carrier inactivation was not translated upon cycloheximide removal.

### DISCUSSION

**Protein Synthesis in Transport Regulation**—Our findings indicate that hexose transport activity of cultured CEF closely correlated with the relative number of functioning hexose carriers in plasma membranes obtained from the cells. Although the mechanisms of transport regulation are not yet elucidated, it appears that the rates of carrier synthesis and inactivation exert a governing effect on transport activity of the cell. Thus, the relative number of functioning carriers appears to be determined by a balance between carrier synthesis and inactivation, such that a change in the activity of one or both mechanisms will lead to enhancement or decline in transport. For example, the increase in transport with glucose starvation could result from greater synthesis activity or decreased inactivation or a combination of the two. Kinetic models based on studies of enzyme synthesis and degradation predict that a decline in the degradative rate results in linear increases of the enzyme, whereas an enhanced rate of synthesis leads to exponential increases of enzyme levels (28). The time course of transport enhancement during starvation approximated an exponential rise (2). However, one of the inherent problems in determining the relative activities of carrier synthesis and inactivation during changes in transport arises when both mechanisms are operating simultaneously.

Previous studies of cultured cells have indicated that carrier synthesis and inactivation mechanisms require protein synthesis, since protein synthesis inhibitors can block the modulation of transport activity during starvation (C→F) or glucose feeding (F→C) (1, 2, 3, 4, 5). Our approach to studying the mechanisms of transport regulation has been to treat CEF with low concentrations of cycloheximide which apparently inhibit the carrier synthesis process while allowing ongoing carrier inactivation. The first indication of a differential effect by cycloheximide on carrier turnover was demonstrated by Christopher et al. (13, 14). They showed that cycloheximide in low concentrations (1–10 μg/ml) elicited a loss of transport in glucose-fed hamster cells, while at high concentrations (>10 μg/ml) there was little change in transport activity. In addition, treatment of starved cells with low concentration cycloheximide was found not to impede the loss of transport upon glucose feeding (S→F + Cx-L), yet high cycloheximide concentrations [S→F + Cx-H] largely pre-

prevent the loss of transport (29). We have confirmed and extended these observations on fed and starved CEF.

Treatment of CEF with low concentrations of cycloheximide (0.5 µg/ml) blunted the increase in transport due to starvation (Fig. 2A), suggesting that carrier synthesis was inhibited. However, during glucose feeding of starved cells (Figs. 1B and 2B), the presence of low concentrations of cycloheximide actually potentiated the decline in transport. Furthermore, fed CEF receiving similar treatment [F + Cyc-L] also experienced significant losses of transport (Figs. 1A and 2A). These findings indicate that low concentrations of cycloheximide did not impede carrier inactivation. However, starved CEF seemed unaffected by low concentrations of cycloheximide since they were able to maintain their elevated level of transport (Figs. 1B and 2B). Therefore, CEF in the starved state appeared to have very low rates of carrier inactivation.

Transport with high concentrations of cycloheximide (50 µg/ml) had no effect on starved CEF and only a modest effect on hexose transport of fed CEF (Figs. 1 and 2). High concentrations of cycloheximide blocked most of the normal enhancement of transport activity due to glucose starvation (Fig. 1B). The slight increase of transport (1.4-fold) during starvation in the presence of cycloheximide may indicate the involvement of a process independent of protein synthesis such as translocation of preformed carriers to the plasma membrane. Such a process, although of a much greater magnitude, has been described for insulin-treated adipocytes (48, 49). It will also be noted that high concentrations of cycloheximide prevented the transport activity of starved cells from declining with glucose refeeding (Fig. 2B). These results occurred during almost total cessation of protein synthesis (as determined by [3H]leucine incorporation, Fig. 2B) suggesting that the inactivation mechanism as well as carrier synthesis are dependent, at least in part, on protein synthesis. Therefore, while low concentrations of cycloheximide seemed predominantly to inhibit carrier synthesis, high concentrations of cycloheximide appeared to inhibit both carrier synthesis and inactivation. The above results have also been obtained with emetine treatment of CEF. We have found that emetine in low concentrations (0.1 µM) appeared to inhibit carrier synthesis and not inactivation, whereas, emetine in high concentrations (10 µM) seemed to block both synthesis and inactivation.

Carrier Inactivation—There are at least two possible mechanisms to account for the finding that carrier inactivation requires protein synthesis. There may be a specific protein directly inactivating the hexose carrier, or one or more nonspecific inactivation mechanisms that require protein synthesis. The present studies provide no evidence for the synthesis of a specific carrier-inactivating protein. Christopher and Morgan have reported that the hexose carrier inactivation mechanism involves lysosomal proteolytic activity (17). There is also evidence that intrinsic membrane proteins are inactivated by a pathway involving internalization and lysosomal degradation (30, 31).

A number of studies have demonstrated that cycloheximide and other protein synthesis inhibitors can block intracellular protein degradation (32–36). There is some evidence that the presence of cycloheximide in high concentrations leads to decreased lysosomal proteolytic activity (37, 38). Cecarini and Eagle have shown that treatment of HeLa cells and diploid human fibroblasts with low concentrations of cycloheximide blocked net protein synthesis, but did not inhibit protein turnover (39). However, at high concentrations of cycloheximide they found that protein turnover was blocked. Our observations that high concentrations of cycloheximide prevent carrier inactivation are consistent with these reports. However, the activity of such a inactivation process is not simply related to the level of cellular protein synthesis, since starved cells manifest very low rates of carrier inactivation yet maintain [3H]leucine incorporation within 80% of the level of fed cells (see "Results"). Thus, while our results indicate a requirement of protein synthesis for carrier inactivation, the actual identity and role of these protein(s) required for this process remain to be elucidated.

Carrier Proteins of CEF Plasma Membranes—Throughout this study hexose transport activity of intact CEF is assumed to reflect the relative number of functioning hexose carrier proteins in the plasma membrane. Although initial rates of influx of 3-O-methylglucose appear to be a reliable measurement of hexose transport (40), this approach has some inherent limitations. For example, a portion of 3-O-methylglucose uptake has been reported to occur by diffusion or some other noncarrier mediated process (4, 41, 42). Therefore, we utilized CEF-derived plasma membrane vesicles to assay d-glucose transport kinetics and [3H]cytochalasin B binding activities. Prior to preparation of plasma membranes, cells were treated in a manner identical with the CEF of Fig. 1B. Both the transport kinetics (Fig. 4) and [3H]cytochalasin B binding activities (Table I) closely correlated with the transport activity determined with intact CEF (Fig. 1B). In addition, we have recently been able to photoaffinity label the components of the presumed hexose carrier of CEF plasma membranes with [3H]cytochalasin B (43). The predominant labeling of fed CEF plasma membranes was of two polypeptides, M, = 52,000 and 46,000, both of which are greatly increased in plasma membranes from starved CEF. D-Glucose transport kinetics, [3H]cytochalasin B binding, and photoaffinity labeling correlated well with the hexose transport activity of intact CEF. Therefore it seems reasonable to assume that the hexose transport activity of CEF reflects the relative number of functioning hexose carriers in the plasma membranes.

Possible Mechanisms of Transport Regulation—The present findings seem to support the hypothesis of Christopher (7) that hexose carriers are regulated by a balance between carrier synthesis and inactivation. There are several possible mechanisms to account for transport regulation. One mechanism often suggested by others is that glucose metabolites have an effect on transcriptional control of carrier synthesis and/or inactivation (1, 4). Some investigators have proposed that metabolites of glucose may stimulate carrier inactivation directly (7, 9, 12) or by stimulating production of mRNA for the inactivation process (4). However, to date there has been no direct evidence that the presence or absence of glucose metabolites exert a regulatory effect on the transcription of mRNA for hexose carrier synthesis or inactivation; nor is there any evidence that glucose metabolites are directly involved in the inactivation mechanism. Starvation could also lead to an increase in transcription of carrier mRNA, as has been suggested regarding the mRNA for glucose-regulated proteins (44, 45). Although glucose starvation has been associated with increased transcription of mRNA for some proteins (46), there is also evidence that during starvation the rate of enhancement of transport is different than the rate of increase in glucose-regulated proteins (47). Furthermore, it would be difficult to explain the differential effects of cycloheximide on hexose transport regulation with a mechanism based simply on changes in the level of transcription.

Another possible mechanism of transport regulation might involve changes in rates of mRNA translation which in turn could result in a differential expression of carrier synthesis and inactivation. This is conceivable since in the case of cycloheximide there is clear evidence that treatment of cells
Hexose Transport Regulation

with low concentrations of the inhibitor (typically 0.2–1 μg/ml) allow translation of some mRNA species more than others (50–55). In addition, hexose starvation has been associated with a decline in the number of polysomes (56, 57) and decreased rates of initiation of translation (58, 59). Since both cycloheximide treatment and glucose starvation seem to have a differential effect on carrier synthesis and inactivation, it is conceivable that translational control may be involved in the regulation of hexose transport. However, it is evident that this and other proposals of transport regulation remain speculative and await further elucidation of the mechanisms involved in hexose carrier synthesis and inactivation.

Acknowledgments—We are indebted to Dr. Alfred L. Goldberg for his valuable advice and review of the manuscript. We thank Virginia Mortaugh for her able technical assistance.

REFERENCES

Regulation of hexose carriers in chicken embryo fibroblasts. Effect of glucose starvation and role of protein synthesis.
K Yamada, L G Tillotson and K J Isselbacher


Access the most updated version of this article at [http://www.jbc.org/content/258/16/9786](http://www.jbc.org/content/258/16/9786)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/258/16/9786.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/258/16/9786.full.html#ref-list-1](http://www.jbc.org/content/258/16/9786.full.html#ref-list-1)