Increased Glucose Uptake Capacity of Rous-transformed Cells and the Relevance of Deprivation Derepression

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ABSTRACT

The increased rate of glucose uptake found in cells transformed by Rous sarcoma virus was shown to be enhanced relative to the changes in uptake induced in nontransformed cells by deprivation of glucose (deprivation derepression). Glucose-specific uptake sites were distinguished from glucose-galactose sites in nontransformed cells, and the capacities for glucose uptake and for galactose uptake were increased to about the same extent by the exclusion of glucose from the cell culture medium. Deprivation derepression occurred without a requirement for new RNA or protein synthesis, suggesting that preexisting inactive uptake sites were activated. Depivation derepression could be mimicked by the treatment of cells with adenosine triphosphatase activators, and adenosine triphosphate levels were reduced in glucose-deprived cells and in cells treated with adenosine triphosphatase activators. Cells transformed by the Bryan strain of Rous sarcoma virus were unresponsive to high concentrations of glucose, to glucose starvation, or to treatment with adenosine triphosphatase activators, and the relative capacity for glucose uptake in these transformed cells was enhanced much more than the capacity for galactose uptake. It was concluded that cells infected by the Bryan strain of Rous sarcoma virus in the process of transformation selectively synthesize more sites specific for glucose uptake. Lower levels of adenosine triphosphate found in transformed cells possibly contribute to a chronic derepression of uptake sites.

INTRODUCTION

An increased rate of glucose uptake is a characteristic feature of CE1 cells transformed by RSV (10, 11, 20). With 3-O-methylglucose, a glucose derivative which is not phosphorylated, the increased uptake was shown to be due primarily to an enhanced entrance of glucose in cells (22, 24). RSV-transformed cells in culture exhibit an increase in V_max for uptake of both 3-O-methylglucose and 2-deoxyglucose (13, 14, 24), indicating that the number of functional uptake sites is greater in these cells than in nontransformed cells. In addition to a higher V_max, cells transformed by the RSV-BH strain of RSV differ from nontransformed cells in a lower apparent K_m for uptake of 2-deoxyglucose (5).

Cells infected with a mutant of RSV-BH, RSV-BH-Ta, have characteristics of RSV-BH-transformed cells at 37° but are nontransformed at 41° (3). Shifting these cells from 41° to 37° results in an increased capacity for deoxyglucose uptake, characterized by an increased V_max and decreased K_m (5). This change in uptake capacity is prevented by inhibitors of RNA synthesis or protein synthesis, indicating a requirement for new transcription and translation in the presentation of new uptake sites to the cell surface.

Nontransformed CE cells deprived of glucose also attain an increased capacity for glucose uptake (17), a phenomenon called "deprivation derepression." The increase can be attributed solely to an increased V_max, since no change in K_m is observed (15). Inhibitors of RNA synthesis and protein synthesis were reported to prevent deprivation derepression, suggesting a classical transcriptional regulation of the glucose uptake mechanism. The similarities of deprivation derepression in nontransformed cells to the enhancement of glucose uptake in RSV-BH-transformed cells suggested a common regulatory mechanism for glucose uptake which would help to explain the malignant character of RSV-transformed cells.

In our experiments on deprivation derepression, inhibition of either RNA synthesis nor protein synthesis prevented the increase in glucose uptake capacity, indicating that the increased uptake capacity of RSV-BH-transformed cells was not the same as deprivation derepression. We also had noticed that the rate of glucose uptake in transformed cells was enhanced to a greater degree than was the rate of galactose uptake (5), while nontransformed cells deprived of glucose increased their uptake capacities coordinately. The results presented here demonstrate that deprivation derepression is exhibited in at least 2 distinct mechanisms for hexose uptake, glucose-specific sites and glucose-galactose sites, both of which may be regulated by cellular ATP levels and neither of which requires new protein synthesis for augmentation. The greater uptake capacity for RSV-BH-transformed cells is primarily due to an increased synthesis of glucose-specific sites, although derepression as seen in nontransformed cells is a constitutive feature also.

MATERIALS AND METHODS

Cell Cultures. CE fibroblasts were prepared and cultured as described previously (5), using modified Eagle's minimal essential medium containing 5% fetal bovine serum, 10% tryptose phosphate broth, and antibiotics. The cells were dispersed and replated at approximately 3-day intervals. Cells plated in 60-mm plastic Petri dishes were used in experiments on the second day after replating before the cells became confluent. Secondary cultures were infected with RSV-BH, RSV-SR, or a mutant of RSV-BH, RSV-BH-Ta (also called td BEIBH), 1 to 10 focus-forming units per cell. Cultures were completely transformed 3 to 6 days following infection. The glucose concentration of the nutrient medium was 11 mM unless otherwise indicated. When cells were to be deprived of glucose, the cultures were rinsed, and Eagle's minimal essential medium without glucose or serum was added. Most deprivation exper-
items were limited to 6 hr, and over this interval the presence or absence of serum was inconsequential.

**Materials.** All radioactive compounds were purchased from New England Nuclear, Boston, Mass.: d-[1-3H]glucose; 2-[3H]-deoxy-α-glucose; 2-[1-14C]deoxy-α-glucose; d-[1-3H]galactose; d-[1-14C]galactose; d-[U-14C]fructose; L-[1-14C]leucine. Cycloheximide and actinomycin D were obtained from Calbiochem, San Diego, Calif.; cordycepin, dinitrophenol, gramicidin, ouabain, ATP, and lyophilized firefly extract were from Sigma Chemical Co., St. Louis, Mo.; puromycin was from Nutritional Biochemicals Corp., Cleveland, Ohio; amphotericin B was from Grand Island Biological Co., Grand Island, N. Y. Oligomycin was a gift from Boehringer-Mannheim, Mannheim, West Germany. Nigericin was a gift from Eli Lilly Co., Indianapolis, Ind.

**Uptake Assays.** Prior to uptake analysis, the growth medium was removed and cells were washed in PBS. Unless concentration effects were under analysis, radioactive compounds were added with nonradioactive homolog to give 20 μM solutions in PBS, and 1 ml was placed in each culture. Cultures were incubated at room temperature (21°) for 5 min, and uptake was terminated by the addition of 5 ml of cold PBS followed immediately by aspiration and extensive rinsing with PBS. Cells were lysed with 1.0 ml of 0.5 N NaOH, and aliquots were taken for scintillation counting or determination of protein (16). Uptake of deoxyglucose and galactose from 20 μM solutions was linear in both transformed and nontransformed cells for at least 20 min (5). Ratios of [5H]deoxyglucose uptake to [14C]galactose uptake were identical at 39° and 21°, although the initial rates of uptake at 21° were about one-half those at 37°.

**ATP Determinations.** ATP was determined by a modification of the luciferase method (1). Semiconfluent cells growing in 100-mm dishes were washed 3 times with cold hypotonic buffer (4 mM MgCl2·50 mM Tris-HCl, pH 7.4), scraped into 3 ml of hypotonic buffer, and transferred to a test tube which was immersed immediately in boiling water. After 90 sec, the material was placed in ice and analyzed within the next hr or stored at −70°. No loss in activity was detected in extracts kept at −70° for up to 3 weeks. Diluted firefly extract was added to a scintillation vial containing cellular extract, both equilibrated to room temperature. The vial was shaken and lowered into the counting well of a Beckman LS 9000 liquid scintillation counter. Counts accumulating during 30 sec were compared to standards prepared from commercial ATP (Sigma).

**RESULTS**

**Deprivation Derepression in CE Cells**

**Effects of Various Sugars on Derepression.** The ability of various sugars to repress (or prevent derepression of deoxyglucose uptake and galactose uptake was examined, with the intention of comparing these results with the ability of the same sugars to compete directly for the uptake of deoxyglucose and galactose. Uptake of both deoxyglucose and galactose increased after glucose was removed from the cultures (Table 1). Glucose and mannose were equally effective in preventing this increase, and 3-O-methylglucose, a glucose derivative which does not become phosphorylated, was partially effective.

Incubation with galactose inhibited the increased capacity for galactose uptake but failed to repress deoxyglucose uptake, while glucosamine and deoxyglucose prevented the increase in deoxyglucose uptake but had less effect on galactose uptake. None of several other sugars (fructose, xylose, fucose, rhamnose, or allose) affected the capacity for deoxyglucose uptake or galactose uptake. These results generally are similar to those described previously for glucose and galactose uptake in CE cells (17) and in mammalian cells (9, 21).

**Competition of Sugars for Uptake Sites.** The same sugars were examined for their ability to interfere directly with deoxyglucose or galactose uptake. The pattern of competition was similar to that of repression; glucose, deoxyglucose, 3-O-methylglucose, glucosamine, and mannose inhibited the uptake of deoxyglucose, and these same compounds plus galactose interfered with galactose uptake. None of the other sugars mentioned above were capable of inhibiting the uptake of deoxyglucose or galactose. The marginal but consistent ability of 3-O-methylglucose to effectively repress deoxyglucose and galactose uptake is seen also in its limited ability to compete with uptake. Also, the failure of galactose to compete effectively with deoxyglucose uptake is correlated with the inability of galactose to repress deoxyglucose uptake. At least 2 possibilities immediately are apparent to explain these results: (a) 2 hexose carriers are present at the cell surface, one of which is relatively specific for glucose uptake and another, present in smaller amounts, which can transport glucose or galactose; and (b) a single carrier transports both sugars, but the carrier has a greater affinity for glucose and other inhibiting sugars than for galactose and noninhibiting sugars.

In an attempt to resolve these possibilities, a kinetic analysis was performed for the uptake of deoxyglucose, galactose, and fructose (Chart 1). The apparent Km for uptake of deoxyglucose in CE cells, 1.0 mM, was similar to that previously reported (5, 13). The apparent Km for galactose uptake was higher, if not incalculable, and fructose (not shown) exhibited unequivocal nonsaturable uptake with the concentration range used in the experiment.

The increase in uptake activity for deoxyglucose, which occurs after deprivation of glucose, has been attributed to an increase in uptake sites since only the Vmax, and not the Km, changes (15). We have confirmed this observation for de-
tose was examined quantitatively in double-labeling experi-
ments. Mixing the sugars did not eliminate the residual noninhibit-
on the total concentration of hexose (galactose plus glucose),
sites. The competition for [3H]galactose uptake was dependent
[3H]galactose to the same extent (Chart 2), but a persistent
ments. Both glucose and galactose inhibited the uptake of
competition for uptake between glucose and galac-
tose was examined quantitatively in double-labeling experi-
ments. Both glucose and galactose inhibited the uptake of
[3H]galactose to the same extent (Chart 2), but a persistent
noninhibitable uptake was found, constituting 50 to 60% of
uncompeted uptake.

Mixtures of glucose and galactose were tested to determine
if glucose-inhibited sites were different from galactose-inhibited
sites. The competition for [3H]galactose uptake was dependent
on the total concentration of hexose (galactose plus glucose),
and mixing the sugars did not eliminate the residual noninhibit-
able uptake. These results suggest that galactose and glucose
were acting competitively on similar sites of galactose uptake,
rather than on 2 dissimilar sites.

Galactose was less effective in inhibiting [14C]deoxyglucose
uptake; in 8 mM galactose, uptake of [14C]deoxyglucose con-
tinued at more than three-fourths the rate of uncompeted
uptake. This suggests that its inhibitory effect on uptake may
occur in a nonsaturable fashion and that galactose uptake occurred through 2
mechanisms, saturable sites and nonsaturable sites.

Reciprocal inhibition of uptake between glucose and galac-
tose was examined quantitatively in double-labeling experi-
ments. Both glucose and galactose inhibited the uptake of
[3H]galactose to the same extent (Chart 2), but a persistent
noninhibitable uptake was found, constituting 50 to 60% of
uncompeted uptake.

Competition for uptake also was examined in cultures de-
prived of glucose for 6 hr, where the rates of uptake of
deoxyglucose and galactose were enhanced about 2-fold. The
relative inhibiting effects of glucose and galactose on [3H]-
galactose uptake and on [14C]deoxyglucose uptake were sub-
stantially increased over the effects in repressed cultures
(Chart 2), suggesting that both the glucose-specific and the
less specific glucose-galactose uptake sites are increased in
deprivation derepression. In consequence, proportionately less
galactose is taken up by the nonsaturable mechanism.

These data were obtained using a 5-min uptake period at
room temperature (21°C). The experiment also was done using
a 15-min uptake interval with results (not shown) essentially
identical to those plotted in Chart 2; i.e., the proportional
inhibition was unchanged by altering the incubation time.
Therefore, it is unlikely that inhibition of uptake by intracellularly
accumulated sugar derivatives is responsible for the results.
Also, the apparent Km for glucose and deoxyglucose uptake
(1 to 2 mM) is more than 10-fold greater than our calculated Km
for hexokinase activity of CE cells, using glucose as substrate
(0.026 to 0.048 mM), suggesting that uptake, and not phos-
phorylation, is the rate-limiting step.

The competitive effects of the glucose analogs deoxyglu-
cose, glucosamine, and 3-O-methylglucose on [3H]deoxyglu-
cose and [14C]galactose uptake were examined (Chart 3). None
of these compounds was as effective as glucose in inhibiting
[3H]deoxyglucose uptake, and their inhibitory activity could be
graded glucose > deoxyglucose > glucosamine > 3-O-methyl-
glucose. In contrast, all were approximately equally effective
in inhibiting [14C]galactose uptake, as indicated in Table 1. 3-
O-Methylglucose inhibited [3H]deoxyglucose and [14C]galac-
tose to about the same extent, suggesting that its inhibitory
activity may be directed toward the glucose-galactose sites,
with little affinity for glucose-specific uptake sites.

The phosphorylated derivatives, glucose 6-phosphate, glu-
cose 1-phosphate, and fructose 6-phosphate, had no signifi-
cant competitive effect on the uptake of [3H]deoxyglucose or
[14C]galactose.

Requirements for Protein or RNA Synthesis. Previous re-
ports indicated that protein synthesis and RNA synthesis were

Chart 1. Lineweaver-Burk plots of deoxyglucose uptake (A) and galactose
uptake (B) in glucose-fed cultures (O) or in cultures deprived of glucose for 6 hr
(C).

Chart 2. Competition of galactose and glucose for uptake of [3H]galactose
and [14C]deoxyglucose uptake in glucose-fed and glucose-deprived cultures. Trace
amounts of radioactive sugars were mixed with various amounts of unlabeled
galactose or glucose, and the uptake of label during 5 min at 21°C was measured.
A, uptake of [3H]galactose into glucose-fed cultures; B, uptake of [3H]galactose
into glucose-deprived cultures; C, uptake of [14C]deoxyglucose into glucose-fed
cultures; D, uptake of [14C]deoxyglucose into glucose-deprived cultures; O, added galactose; O, added glucose; O, added one-half galactose plus one-half
glucose.
required for deprivation derepression (6, 15). We examined the effects of several treatments known to interfere with RNA or protein synthesis. Actinomycin D (2 μg/ml) and high levels of adenosine (5 mM) prevent RNA synthesis by inhibition of transcription and by pyrimidine starvation, respectively. Cordycepin (3'-deoxyadenosine, 0.1 mM) is incorporated into RNA during transcription resulting in defective RNA. These treatments had been tested previously in this laboratory for efficacy in CE cells, and all experiments were limited to a time period where toxic effects were not yet apparent, unless otherwise indicated. None of the treatments prevented the increases in deoxyglucose uptake or galactose uptake induced by glucose starvation (Table 2).

Both cycloheximide and puromycin, inhibitors of protein synthesis, decreased the capacity of glucose-fed cells for deoxyglucose and galactose uptake but failed to prevent increases in uptake upon removal of glucose (Table 2). These results with inhibitors of RNA and protein synthesis demonstrate unequivocally that neither RNA nor protein synthesis are required for the increases in hexose uptake observed in deprivation derepression.

Effects of ATPase Activators on Uptake. Fagan and Racker (7) reported an increased rate of 3-O-methylglucose uptake in cells treated with ATPase activators. The possibility was considered that decreased ATP resulting from glucose deprivation could result in increased uptake capacity, and a variety of compounds likely to affect intracellular ATP were examined for their effects on hexose uptake. Ouabain is a specific inhibitor of Na⁺-K⁺-ATPase, and addition of ouabain to glucose-fed cells decreased the rates of uptake of both deoxyglucose and galactose (Table 3). The noted greater decrease in deoxyglucose uptake compared to galactose uptake was a consistent finding. Ouabain did not prevent derepression, although the magnitude of increased uptake resulting from glucose deprivation was not as great in ouabain-treated cultures as in the control.

Dinitrophenol and oligomycin, both of which decrease mitochondrial ATP formation, increased deoxyglucose and galactose uptake in treated cells. Cells deprived of glucose and exposed to dinitrophenol or oligomycin exhibited deleterious morphological changes within a few hr, presumably due to the total inability of cells to generate ATP either by glycolysis or by respiration.

Increased intracellular Na⁺ might be expected to stimulate Na⁺-K⁺-ATPase resulting in a decrease of intracellular ATP. Gramicidin and amphotericin B alter the general permeability of cells to ions, allowing an increased diffusion of Na⁺ and K⁺ across the surface membrane; nigericin is more selective in allowing an increased Na⁺ permeability. Exposure of glucose-fed cells to these compounds induced an increased capacity for deoxyglucose and galactose uptake (Table 3). No further increase was generated by depriving the cells of glucose, suggesting that ATPase activators and glucose deprivation act in a common way to enhance hexose uptake. The increases in deoxyglucose uptake and galactose uptake produced by treatments with ATPase activators were coordinated; i.e., the relative increase in the rate of deoxyglucose uptake was similar to the relative increase in galactose uptake.

The pattern of leucine uptake (not shown) was different from that of hexose uptake. No increase in leucine uptake was observed after any of these treatments. Decreased uptake was

### Table 2

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<th>Treatment</th>
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<th>[¹C] Galactose</th>
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### Table 3

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<th>[¹C] Galactose</th>
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<td>Oligomycin</td>
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<td>T</td>
</tr>
<tr>
<td>Gramicidin</td>
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<td>T</td>
</tr>
<tr>
<td>Amphotericin B</td>
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</tr>
<tr>
<td>Nigericin</td>
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* T, toxic cells disintegrating.

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**Glucose Uptake in Rous-transformed Cells**

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**Chart 3.** Uptake of [³H]deoxyglucose in the presence of added glucose (△), deoxyglucose (□), glucoseamine (▲), or 3-O-methylglucose (●).
found in cells treated with ouabain, gramicidin, and amphotericin B, consistent with known effects of decreases in intracellular K⁺. Nigericin, dinitrophenol, and oligomycin, at the concentrations shown, had little effect on leucine uptake.

Ouabain and dinitrophenol had antagonistic effects on uptake (Table 4); the repression of hexose uptake by ouabain was overcome by the addition of dinitrophenol, but the full induction of increased uptake capacity found with dinitrophenol alone was not attained. The result is consistent with the anticipated inhibition of Na⁺-K⁺-ATPase by ouabain and with activation of mitochondrial ATPase by dinitrophenol. In nigericin-treated cells, in which Na⁺-K⁺-ATPase would be activated by increased Na⁺ uptake, the stimulation of hexose uptake capacity was prevented by ouabain. The results obtained with mixed inhibitors, therefore, are consistent with the anticipated actions of the individual inhibitors.

Inhibition of protein synthesis by cycloheximide had no effect on either the repression of uptake by ouabain or the induction of uptake by dinitrophenol and nigericin (Table 4).

The kinetics of deoxyglucose uptake was examined after treatment of cells with ouabain, dinitrophenol, and gramicidin. As found after derepression, no change in Kₘ for uptake was detectable; only Vₘₐₓ changed.

**ATP Levels of Treated Cells.** The results of experiments with compounds known to affect ATP suggested that both glucose-specific and glucose-galactose uptake mechanisms were regulated by the intracellular concentration of ATP. Direct measurement of ATP levels showed that glucose deprivation resulted in a decrease in ATP (Table 5). Dinitrophenol and oligomycin, both of which increased hexose uptake capacity, also decreased ATP levels. However, the anticipated increase in ATP expected from the inhibition of Na⁺-K⁺-ATPase by ouabain was not realized; ouabain decreased ATP levels in glucose-fed cells, as well as in glucose-deprived cells, where the concentration of ATP fell to less than one-fourth that of the control.

**Studies on RSV-transformed Cells.** Cells transformed by various strains of RSV exhibit increased rates of glucose uptake compared to nontransformed cells, and the deoxyglucose uptake capacity of RSV-BH-transformed cells was greater than the capacity of RSV-SR cells (Chart 4). The high rates of uptake of deoxyglucose and galactose observed in RSV-BH cells were not enhanced by removal of glucose from the medium (Chart 4A; Table 6), and increasing the glucose in the medium had a minimal repressive effect for uptake in these cells. However, cells transformed by RSV-SR exhibited a slightly enhanced uptake after glucose deprivation and some repression at high glucose levels.

When uptake of deoxyglucose and galactose are assessed simultaneously and compared, it is clear that the increased rate of uptake of deoxyglucose in RSV-BH cells is substantially greater than is the increased rate of galactose uptake (Chart 4B). The relatively high ratio of deoxyglucose uptake to galactose uptake, compared to that of nontransformed cells, was found regardless of the concentration of glucose in the culture

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**Table 4**  
*Induction of uptake by ATPase activators*

Cells were incubated in glucose-containing medium and tested for uptake as described in Table 3.

| Treatment | ³H|Deoxyglucose | ¹⁴C|Galactose |
|-----------|---------------|-----------------|-----------------|
| None      | 1.0           | 1.0              |
| Ouabain   | 0.36          | 0.62             |
| Dinitrophenol | 2.1          | 2.0             |
| Nigericin | 1.7           | 1.6              |
| Ouabain + dinitrophenol | 1.3          | 1.3             |
| Ouabain + cycloheximide | 0.40          | 0.72            |
| Dinitrophenol + cycloheximide | 0.32          | 0.58            |
| Nigericin + cycloheximide | 1.8          | 1.8             |
| Cycloheximide | 1.5          | 1.3             |

**Table 5**  
*ATP levels in treated cells*

Cells in medium with or without glucose were exposed to ouabain (2.5 x 10⁻⁵ M), dinitrophenol (0.2 mM), or oligomycin (10⁻⁵ M) for 6 hr. Cells were rinsed and assayed for ATP.

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<td>None</td>
<td>17.1</td>
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</tr>
<tr>
<td>Ouabain</td>
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<td>Dinitrophenol</td>
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<tr>
<td>Oligomycin</td>
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</table>

**Chart 4.** Deoxyglucose uptake in cells transformed by RSV. CE cells (○) or cells infected with RSV-BH (●), RSV-SR (◆), or a mutant inducing temperature-dependent transformation, RSV-BH-Ta (▲, 37°; □, 41°), were incubated with medium without glucose, or containing 2 or 20 mM glucose for 6 hr. Uptake of ³H|Deoxyglucose and ¹⁴C|Galactose was measured simultaneously; A, uptake of ³H|Deoxyglucose; B, ratio of uptake of deoxyglucose to uptake of galactose. The ratio of ³H|Deoxyglucose to ¹⁴C|Galactose in the uptake medium was set arbitrarily at 1.0.

**Table 6**  
*Hexose uptake in RSV-BH-transformed cells treated with various drugs*

Cells transformed by RSV-BH were exposed to ouabain (10⁻⁴ M), dinitrophenol (0.2 mM), oligomycin (10⁻⁵ M), nigericin (2 µg/ml), gramicidin (10⁻⁵ M), amphotericin B (5 µg/ml), or dimethyl sulfoxide (2%, v/v) for 6 hr prior to measuring the rates of uptake of ³H|Deoxyglucose and ¹⁴C|Galactose.

<table>
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<tr>
<th>Treatment</th>
<th>Glucose</th>
<th>Glucose</th>
</tr>
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<tbody>
<tr>
<td>None</td>
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<tr>
<td>Ouabain</td>
<td>0.27</td>
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<td>Dinitrophenol</td>
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<td>Nigericin</td>
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<td>Gramicidin</td>
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<td>Amphotericin B</td>
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<td>Dimethyl sulfoxide</td>
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The kinetics of deoxyglucose uptake were examined after treatment of cells with ouabain, dinitrophenol, and gramicidin.
Glucose Uptake in Rous-transformed Cells

medium and irrespective of the concentration (below 0.1 mM) of either sugar in the uptake medium or the isotopic label on the sugar. A similar observation was made in cells infected with a mutant of RSV-BH, RSV-BH-Ta, which induces transformation at 37° but not at 41°. Cells maintained at the transformation-permissive temperature had higher uptake ratios of deoxyglucose to galactose than did cells maintained at 41°. Cells transformed with RSV-SR had uptake ratios of deoxyglucose to galactose intermediate between nontransformed cells and RSV-BH-transformed cells, consistent with the repression effects cited above.

These results suggested that in RSV-BH-transformed cells the glucose-specific uptake mechanism had increased to a greater extent than had the glucose-galactose uptake mechanism. In the same experiment described previously in Chart 2, competition for radioactive deoxyglucose and galactose uptake by unlabeled glucose and galactose was examined. Galactose at concentrations up to 8 mM had no significant inhibitory effect on deoxyglucose uptake (Chart 5), suggesting that virtually all of the deoxyglucose was taken up by glucose-specific sites. As in nontransformed cells, glucose at 8 mM competed for less than one half of the galactose uptake, suggesting that non saturable sites were still responsible for a substantial portion of galactose uptake. The pattern and degree of heterologous competition was unchanged by increasing the uptake interval from 5 to 15 min.

Homologous competition, however, was increased by expanding the uptake time, both with glucose versus [14C]deoxyglucose and galactose versus [3H]galactose (Chart 5). These results suggest that higher intracellular concentrations of glucose and galactose resulting from increased uptake rates overload the available hexokinase and galactokinase, respectively, and that, at concentrations of either sugar above about 0.2 mM, membrane transposition is no longer rate limiting.

Effects of Compounds Altering ATP. In contrast to nontransformed cells, treatment with compounds known to affect ATP failed to stimulate the capacity for deoxyglucose uptake in RSV-BH-transformed cells (Table 6). Dinitrophenol and oligomycin had no significant effect, while nigericin, gramicidin, and possibly amphotericin B decreased the rate of deoxyglucose uptake.

Ouabain and dimethyl sulfoxide both decreased the capacity for deoxyglucose uptake when cells were grown in the presence of glucose, but when glucose was omitted from the medium these decreases were not observed. Ouabain had a lesser effect on galactose uptake in the same cells, and di methyl sulfoxide had no inhibitory activity, whether or not glucose was provided. Therefore, glucose in the presence of ouabain or dimethyl sulfoxide had a selective suppressive effect on glucose-specific uptake sites.

As reported previously (4), ouabain has no direct or immediate effect on deoxyglucose uptake. When cells were incubated for several hr in ouabain, inhibition was dependent upon the duration of exposure (Chart 6). The selective effect of ouabain on deoxyglucose uptake compared to galactose uptake was more evident in RSV-BH cells than in nontransformed CE cells, and by 6 hr the ratios of deoxyglucose uptake to galactose uptake in the 2 cell lines were similar.

ATP Levels in Transformed Cells. Levels of ATP in RSV-BH-transformed cells and nontransformed cells were compared (Table 7). Transformed cells characteristically exhibited lower amounts of ATP than did nontransformed cells. Neither ouabain

<table>
<thead>
<tr>
<th>Cells</th>
<th>ATP levels in RSV-BH-transformed cells (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE</td>
<td>12.0</td>
</tr>
<tr>
<td>RSV-BH</td>
<td>5.5</td>
</tr>
<tr>
<td>RSV-BH + ouabain</td>
<td>1.8</td>
</tr>
<tr>
<td>RSV-BH + dimethyl sulfoxide</td>
<td>4.4</td>
</tr>
<tr>
<td>RSB-B - glucose</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Chart 6. Changes in relative uptake of deoxyglucose and galactose in cells treated with ouabain. CE cells (○) and RSV-BH-transformed cells (△) were exposed to ouabain (10^{-4} M) for various times prior to the simultaneous measurements of rates of uptake of [3H]deoxyglucose and [14C]galactose. The uptake ratios are calculated after setting the [3H]/[14C] ratio of the uptake medium equal to 1.0.
nor dimethyl sulfoxide raised the level of ATP after treatment for 6 hr. In contrast, ouabain substantially decreased the intracellular ATP in transformed cells, as in nontransformed cells. A slight decrease in ATP was found in RSV-BH cells deprived of glucose during this interval.

DISCUSSION

The results presented here suggest that glucose uptake in CE cells occurs mainly via 2 mechanisms, a glucose-specific mechanism which is responsive to competition by glucose, but not galactose, and another mechanism which can be affected by glucose or galactose. Galactose transport occurs through the glucose-galactose sites and through nonsaturable sites which are unaffected by high concentrations of sugars. These separate mechanisms for uptake were indicated in earlier experiments (5), in which the cellular capacity for deoxyglucose uptake, but not galactose uptake, was markedly influenced by the pH of the culture medium.

The sugars which effectively compete with radioactive deoxyglucose for uptake (glucose, mannose, deoxyglucose, and glucosamine) are the same as those which prevent the increased capacity for deoxyglucose uptake resulting from glucose deprivation. Likewise, the lesser competitive ability of galactose and 3-O-methylglucose for deoxyglucose uptake is seen also in the lesserened ability of these sugars to prevent deprivation derepression for deoxyglucose uptake. These correlations suggest that repression and derepression are functions of the uptake sites and are affected in some direct way by the sugars which utilize these sites.

Deprivation derepression is accompanied by an increase in the Vmax for deoxyglucose uptake, without a change in Km, suggesting an increase in the number of transport sites in the cell surface membrane. The failure of inhibitors of protein or RNA synthesis to prevent the deprivation derepression in our experiments with CE cells, and as reported for hamster cells (6, 8), argues against a role for transcription or translation in this phenomenon. Other conflicting reports (8, 15) perhaps can be explained by the differences in physiological state of the cells or confluency of cells in the cultures, conditions which are known to be major factors in glucose uptake. These latter results were interpreted as evidence for synthesis of new transport sites, whereas our results suggest that inactive or cryptic sites become activated or exposed as a result of glucose deprivation.

Treatment of cells with dinitrophenol or oligomycin resulted in decreased intracellular ATP levels and an increased uptake capacity (7, 12) similar to that found in deprivation derepression. With ATPase activators and with glucose deprivation, the relative increase in deoxyglucose uptake capacity is about the same as the relative increase in galactose uptake capacity, suggesting that both the glucose-specific uptake sites and glucose-galactose uptake sites are activated by these treatments. As in deprivation derepression, cycloheximide failed to prevent increases in hexose uptake induced by ATPase activators.

Increased uptake resulting from treatment of CE cells with ATPase activators suggested that glucose-specific and glucose-galactose sites are regulated by the availability of ATP. These compounds, in fact, decreased the ATP levels of treated cells, as did removal of glucose from the cell culture medium. While a role for ATP in deprivation derepression is hard to ignore, the correlation is incomplete, since ouabain, an inhibitor of Na1K+-ATPase, decreased rather than increased, ATP levels.

The increases in glucose uptake capacity seen in RSV-BH cells differ from those of deprivation derepression or ATPase activation. The increase in uptake occurring during transition from the nontransformed to transformed phenotype in a temperature-dependent system can be prevented by inhibitors of RNA or protein synthesis, demonstrating that macromolecular synthesis is required for the increased uptake capacity (3). In RSV-BH-transformed cells, a decrease in Km is observed, as well as an increased Vmax (5), and these differences were found in the temperature-dependent transformation system as well. It should be noted, however, that the lower Km of RSV-BH-transformed cells may reflect the fact that hexokinase has become rate limiting, rather than uptake, because this was suggested by the ability of high levels of glucose to compete more effectively with radioactive deoxyglucose uptake if the incubation time for uptake was extended. Therefore, RSV-BH cells synthesize more glucose-specific sites which may be qualitatively similar or identical to those of nontransformed CE cells.

The synthesis of glucose-specific sites is supported by the observation that the capacity for glucose (or deoxyglucose) uptake in RSV-BH cells is substantially enhanced relative to the capacity for galactose uptake. On the other hand, both ouabain and dimethyl sulfoxide markedly reduced the ability of these cells to transport deoxyglucose, while the uptake of galactose was affected much less. These observations indicate that the synthesis of glucose-specific sites is selectively induced during transformation by RSV-BH.

The failure of high concentrations of glucose to repress the capacity for glucose uptake in RSV-BH cells suggests either that the new sites are nonrepressible or that all glucose and glucose-galactose sites are constitutively repressed in RSV-BH cells. We have not attempted to distinguish between these possibilities.

Salter and Weber (19) have shown that the higher glucose uptake capacity of RSV-transformed cells is paralleled by an increase in binding of cytochalasin B and that glucose could compete with cytochalasin B for the binding sites. Although termed "glucose specific," the relative ability of galactose to compete with cytochalasin B binding was untested, and the possibility remains that cytochalasin B binds to both glucose-specific and glucose-galactose sites, as defined here.

RSV-BH-transformed cells also contained lower levels of ATP than do nontransformed CE cells, which may be responsible for the nonrepressibility of uptake sites. Nonetheless, the lowered ATP levels and concurrent derepression are insufficient to explain the greater uptake capacity for glucose exhibited by transformed cells. The lower ATP level of RSV-BH-transformed cells may explain the occurrence of lower cyclic adenosine 3'5'-monophosphate levels reported for these cells (18). Other properties of RSV-BH-transformed cells, e.g., increased thymidine uptake (5) or decreased alkaline phosphatase activities (2), are not found in cells deprived of glucose or treated with dinitrophenol, and it seems unlikely that small differences in intracellular ATP concentration contribute in a

1 Unpublished observations.
major way to the malignant potential or phenotype of RSV-BH-transformed cells.

The selective synthesis of glucose-specific uptake sites in RSV-transformed cells may be an important factor in the malignant potential of these cells. The increased aerobic glycolysis exhibited by these cells (20), and considered by Warburg (23) and others to be a determinative property of tumor cells, can be explained by the increased capacity for uptake of glucose. Also, the substantially increased production of hyaluronic acid by RSV-transformed cells (3) may be due to the increased intracellular availability of glucose metabolites. While these and other properties of the transformed cells (e.g., glycosylation differences) may be related to increased glucose uptake, and relevant to the malignant phenotype, it is important to note that the viral gene product directly responsible for transformation has functions which precede the transcriptional induction of these activities in RSV-infected cells (3, 5).

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Increased Glucose Uptake Capacity of Rous-transformed Cells and the Relevance of Deprivation Derepression

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