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Carbohydrate Catabolism and the Enhancement of Uptake of Galactose in Hamster Cells Transformed by Polyoma Virus*

(tumor transformation/nonglycolytic hexose/accumulation of galactitol)

H. M. KALCKAR, D. ULLREY, S. KIJOMOTO, AND S. HAKOMORI

Biochemical Research Department, Massachusetts General Hospital and the Department of Biological Chemistry, Harvard Medical School, Boston, Mass. 02114; and the Departments of Pathobiology and Microbiology, University of Washington, Seattle, Wash. 98195

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ABSTRACT **Untransformed as well as polyoma virus-transformed hamster cells can be grown equally well on a slow catabolite like galactose as on a rapid catabolite like glucose. The rate of uptake of galactose is greatly enhanced in the transformed cells as compared with untransformed cells, and this enhancement of entry was as markedly expressed in galactose-grown cultures as in glucose-grown cultures. Since the transformed cultures grown in glucose medium consume practically all of their carbohydrate, contrary to the galactose-grown cultures, problems dealing with regulation of transport by substrate concentrations have to be dealt with also. The galactose captured by the cells accumulates initially as galactose, α-galactose-1-phosphate, and UDP-galactose. However, after a 24-hr growth on a galactose growth medium, the product accumulated was almost exclusively galactitol. In spite of the enhancement of entry of galactose into the transformed cells, the metabolic pathway becomes stalled even before it has reached the stage of glucose-1-phosphate, largely due to a choke of the enzyme UDP-galactose-4-epimerase (EC 5.1.3.2). Among the sparse amounts of catabolic products generated by the transformed cells from galactose, carbon dioxide (albeit no D-xylose) and lactic acid were found, both of them in much smaller amounts than seen if glucose is the carbohydrate source. Also, growth of transformed cells on a galactose medium gradually tends to become oriented, a phenomenon that could be called "contact promotion." Subsequent addition of glucose disturbs the oriented growth and interferes with contact promotion.**

Human cell lines such as HeLa that grow on glucose growth media show high aerobic glycolysis (1, 2). Replacement of glucose by galactose permits an appreciable growth rate (about one third of that on a glucose growth medium), yet only minute amounts of lactic acid are formed, presumably because galactose is utilized more efficiently through an oxidative pathway (1, 2).

Later studies on L cells (3) or mammary cells in dedifferentiation (4) indicated that the activity of the enzyme UDP-galactose-4-epimerase (EC 5.1.3.2) in such cultures was extremely low, below the point of detection (3, 4). It is known that NAD is required for the activity of mammalian epimerase (5) and that NADH is a strong inhibitor. In fact, crude, undialyzed lysates of human erythrocytes do not show any detectable epimerase activity until NAD is added (6, 7). Likewise, in more recent studies of epimerase activity in crude, broken L- and HeLa-cell preparations, epimerase activity was readily detectable provided a large excess of NAD was added to the preparation (8, 9). In intact L cells or HeLa cells, however, the epimerase activity was barely detectable and was only a minute fraction of that found in broken cells incubated with excess NAD (8). Hence, the cellular milieu in these cases exerted a powerful feedback inhibition on mammalian epimerase. We have found the NADH/NAD ratio and the pH, and especially the combination of both, to be the main factors (8, 9) in this severe curtailment of epimerase activity and thus also of galactose catabolism.

In the present article, we describe studies on transport and metabolism (especially catabolism) of galactose in hamster-cell cultures untransformed (BHK, NIL) as well as transformed by polyoma tumor virus (BHKpy, NILpy).

It has been found that transformation by tumor viruses markedly stimulates transport of glucose, 2-deoxyglucose, and galactose into cultured cells (10–13). The enhanced rate of transport of glucose into transformed cells may be responsible for the rapid catabolism and, especially, the high aerobic glycolysis of glucose in these cells.

However, a recent examination of transport processes of glucose in avian cell cultures by Amos and coworkers (14) has raised some new problems. It was shown that the rate of uptake of glucose (or 2-deoxyglucose) by chick fibroblast cultures is governed to a surprising degree by the glucose concentration in the medium during growth. High glucose concentrations during growth seem to "repress" glucose transport, whereas low concentrations elicit a "depression" effect on this transport system. Since tumor virus-transformed cell cultures, by their heavy consumption of glucose, reduce the concentrations rapidly, the "derepression" phenomenon, if it were operating in such cultures, might well be involved in the enhanced transport of glucose (or 2-deoxyglucose) found in tumor cells.

As will appear from this article, BHK cultures transformed by polyoma virus (BHKpy), which have a very rapid consumption of glucose, consume galactose much more slowly than glucose, during both incubation and growth. Amos and coworkers have shown, in the same study of avian cells, that although galactose does not "repress" glucose transport, both glucose and galactose exert a distinct, albeit a more moderate, "repression" effect on galactose transport (14).

Accordingly, we have varied the source of carbohydrate during growth of untransformed and transformed hamster cells in order to examine galactose transport as a function of

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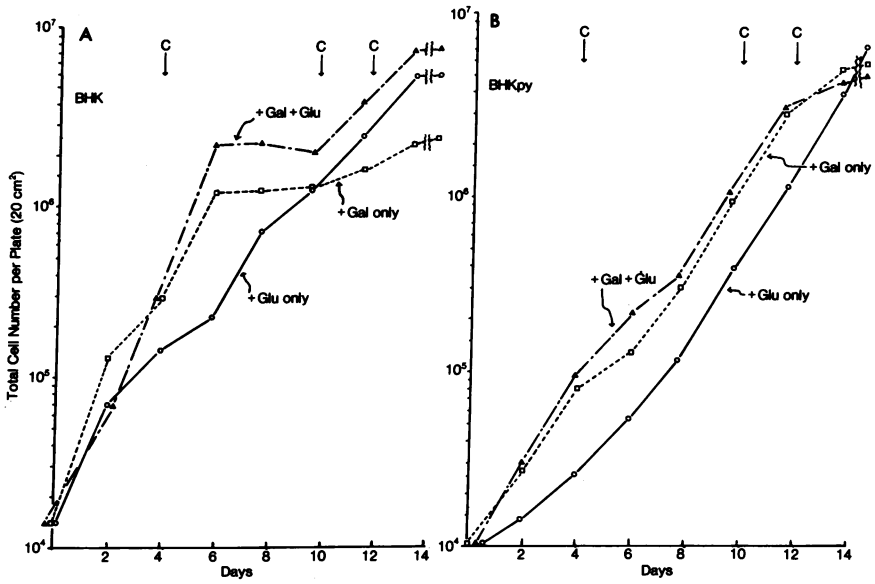

FIG. 1. Growth curve for untransformed BHK cells (*A*) or transformed BHKpy cells (*B*) in growth medium containing either 4.5 μmol/ml of glucose or 4.5 μmol/ml of galactose or both (2.2 μmol/ml of each). (*C*) Change of culture medium.

hexose consumption as well as tumor transformation. Since galactose is able to sustain excellent growth of untransformed as well as transformed hamster cells, this sugar seems to serve as a highly useful carbohydrate source.

MATERIALS AND METHODS

The BHK cell lines stemmed from Dr. M. Stokers laboratory and were donated by Dr. I. Macpherson. NIL cells (15) were donated by Dr. Leila Diamond. Both BHK and NIL cells were transformed in this laboratory by polyoma virus, and the transformed clones were isolated by the agar selection method (16). The effect of galactose on the growth behavior of py (transformed) cells was observed within 30 passages after transformation. The cells were grown in a plastic Falcon petri dish with Eagle's medium (MEM-ES, Assoc. Biomedic Systems Inc., New York, N.Y., p. 8) reinforced 3 times with vitamins and amino acids and buffered by bicarbonate, phosphate (see MEM-ES), and 5% CO_2 atmosphere. Fetal-calf serum, undialyzed or dialyzed, was added up to 10%. Before contact inhibition ensued, the density of BHK cells corresponded to 8×10^6 cells per bottle, or 2.8 mg of protein. BHKpy transformed cells amounted to 2–4 times more cell protein per bottle. The cell density was 1.2 to 1.5×10^7 cells per bottle (20 cm²); the volume of growth medium was 8 ml. The carbohydrate sources used were either glucose or galactose, nonlabeled or single- or double-labeled. The exact concentration of glucose or galactose in the growth medium after addition of serum at the start of the growth experiment was 4.5 μmol/ml. [*U*-^{3}H]Galactose, [*1*-^{3}H]galactose, [*1*-^{14}C]-galactose and [*2*-^{14}C]glucose (NEN) were used either during growth or in the 1-hr incubation. In the former case, the radioactive sugars were added to the hexose-free growth medium (MEM-ES), in the latter case, to the phosphate-buffered saline pH 7.4 (Assoc. Biomedic Systems, Inc. p. 6). The hexose concentrations used were 4.5 μmol/ml except in one incubation experiment in which it was 9-times lower.

For determination of growth curve and behavior, cells were plated in several petri dishes of 5-cm diameter, and cell numbers in each of four petri dishes were counted every 12 hr by a Coulter counter; the average number was recorded. Growth behavior, cell orientation, and change of morphology were observed on cultures in petri dishes. In a separate experiment, in order to minimize the effect of pH change, a relatively small number of cells (10^6) was grown on a small glass cover slip (diameter 1.5 cm), placed in a petri dish (10-cm diameter) with 12 ml of medium.

The cells were rapidly fixed in 70% ethanol. The soluble fraction (ethanol supernatant) was concentrated and chromatographed (8). Separate analyses of labeled UDPGal and UDPG were performed by two different methods. The first method was 2-dimensional chromatography, with polyethyleneimine on thin-layer plates and LiCl and borate solution (16). The same technique was used with ^{3}H- and ^{14}C-labeled galactose. The second chromatography method uses specific enzymes (8), the action of which converts UDPG to UDPGlucuronate, which moves slower than UDPG and UDPGal both in one- and 2-dimensional chromatography of the type used. Galactose and Gal-1-P were analyzed before and after acid hydrolysis by use of the specific galactose dehydrogenase and NAD (8). The galactose concentrations in the medium and in the cells were determined by chromatography and counting, as well as by the galactose dehydrogenase assay by measurement of the NADH formed by spectrophotometry or spectrofluorometry (Ullrey, Kalckar, 1972, unpublished).

Lactic acid was determined by use of lactate dehydrogenase and NAD; the NADH formed was assayed by spectrophotometry or spectrofluorometry. Lactic acid stemming from labeled hexoses was determined by paper chromatography [3 volumes of 1 M ammonium acetate–acetic acid (pH 3.8) and 7.5 volumes of 95% ethanol]; the small fast-moving peak was eluted and counted (Ullrey, Kalckar, 1972, unpublished).

RESULTS

The rate of uptake of labeled galactose into BHK cells was assessed as the amount of labeled galactose and Gal-1-P

that accumulates, after 1 hr of incubation in growth medium or phosphate-saline medium at 37°.

Table 1 summarizes the results of several experiments in which the rate of uptake of labeled galactose by BHK (or NIL) cells was compared with that of cells transformed by polyoma virus (BHKpy or NILpy). It can be seen that py transformation of the cell population brings about a 2.5- to 3-fold stimulation of the rate of uptake of galactose over a 1-hr period. Moreover, this enhancement of galactose transport is independent of the source of the carbohydrate used during the growth period. There is no indication of any induction or repression of galactose transport by the administration of galactose as the main carbohydrate source during the growth period; nor did we find an increase of galactokinase activity in the py transformed hamster cells that could account for the marked enhancement of galactose entry (Hakomori and Kalckar, 1972, unpublished).

Finally, Table 1 also illustrates that the increased rate of galactose transport in the py cells is not accompanied by any relief of the epimerase choke, rather the choke may be intensified in some cases (see UDPGal/UDPG ratios in column 4 of Table 1 in which ratios larger than 5 are frequently encountered in the py series).

TABLE 1. *Enhancement of galactose entry by py; accumulation of UDP galactose.*

Hamster fibroblasts normal/py	Pregrown on galactose or glucose	Accumulation of labeled galactose compounds, nm/mg of cell protein per hr	Ratio of hexose-labeled UDPGal over UDPG
BHK norm	Glu	8.3	2.0
BHK py	Glu	18.3	3.7
BHK norm	Gal	10.0	1.6
BHK py	Gal	40.0	4.5
BHK* py	Gal	20.8	6.8
BHK py	Gal	20.0	
BHK py	Gal†	19.0	
NIL norm	Glu	11.8	1.5
NIL py	Glu	27.5	4.0
NIL norm	Gal	5.8	2.8
NIL py	Gal	24.2	16.7
NIL* py	Gal	29.0	4.0
NIL spontaneous	Glu	19.2	2.1
NIL spontaneous	Gal	18.3	2.8

Growth conditions: 8 ml of growth medium with 10% fetal-calf serum (undialyzed or in one case dialyzed); glucose or galactose concentrations were 4.5 μmol/ml. After washing with phosphate-buffered saline, the cells were suspended in 1 ml of the buffered saline containing radioactive galactose (see *Methods*) in a concentration of 4.5 μmol/ml. Incubation was at 37° for 60 min. For analyses, cells were washed at 0° with 3 ml of phosphate-buffered saline, spun, and then treated with 70% ethanol at 45–50° for 2–3 min while stirring. After spinning, the alcoholic supernatant was counted and the radioactive material accumulated was assayed from the specific radioactivity of the galactose of the medium. The cells did not contain any significant pool of galactose or gal-1-P.

* Cells oriented.

† Grown in the complete absence of glucose, i.e., fetal-calf serum dialyzed.

TABLE 2. *Galactose metabolites in BHKpy cells after 24-hr growth with [^{14}C]galactose* and 1-hr incubation with [^{3}H]galactose*

Location	Paper chromatography: cpm ^{14}C	cpm ^{3}H	Ratios ^{14}C/^{3}H
Origin	266	2	
UDPGA	970	654	1.48
UDPGHexose	1,586	2,468	0.64
Gal-1-P	1,236	986	1.25
"Gal"	10,118	1,674	6.04
"Lactate"	228	274	0.83

* If cell densities did not exceed 1.2×10^7 cells per bottle (75 cm^2), only 20–30% of the 36 μmol of galactose in the 8 ml of growth medium was consumed.

As a rule, the amount of galactose accumulated in the cells was somewhat higher than that of Gal-1-P. However, the amount of UDPHexose was also appreciable. A slow-moving spot corresponding to the position of UDPGlucoronate comprised only a small fraction. However, if it is UDPGlucuronate, it should be added to the UDPG denominator in the UDPGal/UDPG ratio, which then becomes smaller, although still larger than 2 to 3 (see *Discussion*).

As compared with glucose, galactose turned out to be a very slow catabolite for hamster cells, even in the py transformed cells, and much less lactic acid was formed. The consumption of galactose was slow during growth (see footnote to Table 2) as well as during incubation after growth (Table 3). From Table 3 it is also clear that carbon dioxide formation

TABLE 3. *Slow catabolism of galactose in BHKpy.*

	[2-^{14}C]Glucose + [U-^{3}H]Galactose: 500 nm/ml (%)	500 nm/ml (%)	^{14}C/^{3}H ratio found
Original hexose mixture	100	100	0.5
Hexose consumption	90	18	
Hexose remaining in medium	10	70	0.09
CO_2 formed (trapped in hyamine)	5	(1.5†)	
Lactic acid formed (mainly in medium)	85	6	6.02
Hexose compounds accumulated in cells		5	0.35
UDPGal in cells			0.02
UDPG in cells			0.08

* 10^7 BHKpy cells (about 3 mg of protein) were suspended in 1 ml of phosphate-buffered saline with 0.5 μmol of glucose and 0.5 μmol of galactose and incubated 60 min at 37° (for further details see footnote to Table 1).

† Separate experiment with [1-^{14}C]galactose.

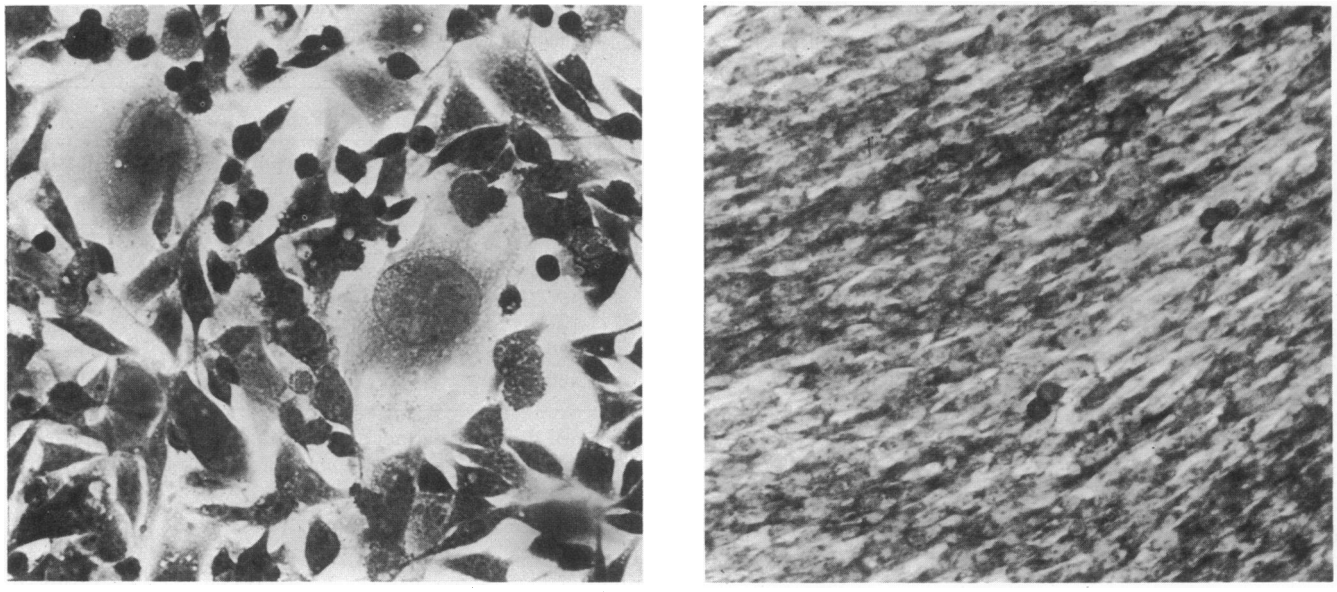

FIG. 2. NILpy cultured 10 days in 0.1% glucose (*left*) or 0.1% galactose (*right*) (4.5 μmol/ml of each). Magnification: ×30.

from galactose was much smaller than from glucose. No xylose formation from [*U*-^{3}H]galactose was discernable.

The galactose cultures so far described have not been grown in the total absence of glucose inasmuch as the growth medium contained undialyzed fetal-calf serum, usually in amounts of 10%, corresponding to 0.01% glucose. Under these conditions, growth on 0.08% galactose was not only as rapid as growth on glucose but surpassed it as illustrated in the growth curves of BHK and BHKpy (Fig. 1). Metabolism and transport of galactose by cells grown on medium with *dialyzed* fetal-calf serum and with pure galactose was slower but showed much the same features (see later in this article).

In untransformed cells galactose permits a pronounced contact inhibition. In transformed cells growth on galactose is excellent, somewhat faster than that of glucose, especially in the sparse cultures. In late growth (i.e., after 10 days or more), the morphology of the BHKpy and NILpy cultures grown in media with galactose frequently changes, becoming oriented in multilayers as contrasted to the disoriented BHKpy or NILpy cultures grown in media with glucose (see Fig. 2). These oriented cultures do not show contact inhibition; rather, they grow very well as multilayers of well-oriented sheets ("contact promotion," see ref. 19). The pH of the culture medium of the galactose-oriented BHKpy or NILpy cells often showed strong shifting towards a range (7.6–7.8) more basic than physiologic. The galactose-oriented cell sheets slowly lose their orientation during the first few days, if the galactose medium is replaced by a regular glucose medium; the orientation immediately disappeared upon subculture of the oriented plate by trypsinization. The galactose orientation was more remarkable in NILpy than in BHKpy cultures and was more readily achieved with freshly transformed cells than with cells many generations after transformation.

TABLE 4. *Further resolution of "Gal" peak from Table 2*

Rechromatography of "Gal" peak on thin-layer chromatography				
		cpm		Ratios
Location	R_F	^{14}C	^{3}H	^{14}C/^{3}H
Galactitol area	0.12	7414	688	20.8
Galactose area	0.53	86	880	0.1
Xylose area	0.70	61	770	0.08

Galactitol rather than Gal-1-P, the terminal accumulation product during growth

In a series of growth experiments of BHKpy on [*1*-^{14}C]-galactose in the presence of dialyzed or undialyzed fetal-calf serum, the types of products that accumulated were different from those found during the shorter incubation period after growth. In some of these experiments, the cultures grown 24 hr on [*1*-^{14}C]galactose were incubated for one additional hour on [*U*-^{3}H]galactose. Paper chromatography of the water-soluble, low-molecular-weight constituents revealed a ^{14}C peak in the galactose region or somewhat more distal. This "Gal peak" was eluted, counted, and rechromatographed with various standards either on DEAE-paper or on silica-gel plates with borate (20). The results were as follows (see Table 2).

The ^{14}C "pseudo galactose" peak migrated actually somewhat faster than the galactose standard on paper chromatography with the ethanol, acetic acid, ammonium acetate mixture at pH 3.5. On DEAE-thin-layer chromatography the unknown ^{14}C-labeled peak ran as fast as galactose, ruling out galactonate. On silica-gel plates with 0.02 M borate (see ref. 20) the ^{14}C-labeled material moved much slower than galactose and the position corresponded to that of galactitol (Table 4). Other areas corresponding to galactose contained no ^{14}C. The ratio ^{14}C/^{3}H in these areas was much lower than that of the Gal-1-P peak from paper chromatography. The formation of galactitol was also pronounced in the cultures grown on dialyzed fetal-calf serum. Thus, the ^{14}C/^{3}H ratio in the crude galactitol peak (from paper chromatography) was 5.2, whereas the ratios for Gal-1-P and UDPHexose were 1.05 and 0.98, respectively (see Tables 2 and 4).

The concentration of galactitol in the cells after 24 hr of growth in medium with galactose reached the 1 mM level.

DISCUSSION

Although it has been shown that cultured cells are able to grow with galactose as the only carbohydrate source (21), it is noteworthy that the BHKpy transformed cells are also able to do so and with a relatively fast growth rate. And yet, the 1-hr incubation tests show that galactose is utilized very slowly with the formation of only small amounts of lactic acid and CO_2. The moderate amounts of CO_2 formed might partly stem from the degradative pathway to D-xylose, although no significant amounts of D-[U-^{3}H]xylose could be detected (and no galactose dehydrogenase activity in broken BHKpy cells was discernable). During a 1-hr incubation, at least one-third of the galactose consumed seems to accumulate in the cell as galactose, Gal-1-P, and UDPGal. From this experiment, which is illustrated in Table 3 and in which a low galactose concentration was used (0.5 μmol/ml), one should be able to extrapolate to the rate of consumption during growth. A rough computation shows that 10^7 cells of BHKpy over 24-hr growth in 8 ml of medium with the usual concentration of galactose would consume only about 20% of the total amount (see also Table 2 footnote).

The role of galactose as a catabolite during growth is not clear either. After 24 hr on growth medium with galactose as the carbohydrate source, the main constituent that accumulated in the cell was galactitol; neither galactose nor Gal-1-P were found in significant amounts. This is reminiscent of observations made on chicken brain at the time neurotoxic symptoms appear after feeding of an extensive amount of galactose (22); at that time galactitol is the dominating product that accumulated.

The tendency towards oriented growth in the 10- to 14-day BHKpy cultures grown with galactose as the main carbohydrate source poses new problems. Among the factors that should be considered, one could list pH (23) or increased steady-state concentrations of cyclic AMP (24, 25), either as a result of a higher pH or other factors such as the accumulation of galactitol. The steady-state concentrations of mucopolysaccharides (26) should also be cited as a potential factor influencing the morphology of cells. It should be mentioned that BHKpy cultures grown on medium with galactose did not transfer any terminal α-galactosyl unit to its lactosylceramide but kept the simple lactosyl pattern characteristic of transformed cells (27–29).

Regarding the enhancing effect of transformation of hamster cells on the rate of galactose entry, the following picture emerges. The effect was observed in the hamster cell cultures grown on glucose (average 2.3) as well as those grown on galactose (average 3.0). As mentioned, galactose consumption is low even in the transformed strains. Hence, if any repression of hexose entry, elicited from unconsumed hexose (see ref. 13 and introduction) has occurred, it did not eliminate the effect of py transformation on galactose entry into hamster cells. It should also be mentioned that Hatanaka had observed earlier (30) that enhancement of glucose entry in mouse-embryo fibroblasts by mouse sarcoma virus was fully expressed even when the glucose concentrations were still high in the transformed cultures.

Our own observations might equally well be explained by a repression effect on galactose entry into untransformed cells grown on galactose medium, if one assumes that the transformed hamster cells are much less responsive to this type of "repression."

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