Two Distinct Types of Enhancement of Galactose Uptake into Hamster Cells: Tumor-Virus Transformation and Hexose Starvation

(UDPglucuronic acid /contact inhibition/polyoma virus)

H. M. KALCKAR AND D. ULLREY

Department of Biological Chemistry, Harvard Medical School and Biochemical Research, Massachusetts General Hospital, Boston, Mass. 02114

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ABSTRACT Enhancement of hexose uptake seems well correlated with transformation of cell cultures by tumor viruses and the absence of contact inhibition. Enhancement of sugar uptake has also been observed as a result of hexose starvation. Both types of enhancement can clearly be demonstrated in cultures of hamster cells when uptake of ^{14}C -labeled galactose is monitored after 10 or 20 min. The profiles of accumulation products are strikingly different. In cultures of hamster NIL cells transformed with polyoma virus much of the ^{14}C is accumulated as UDPhexose. Untransformed cells accumulate galactose-1-phosphate as well as UDPhexose. Hexose-starved cells show enhanced uptake of galactose; however, this marked enhancement was only observed in NIL cultures close to contact inhibition. The novel and common feature seen in hexose-starved cells when incubated briefly with ^{14}C -labeled galactose is the occurrence of a marked accumulation of [^{14}C]UDPglucuronic acid at the expense of UDPhexose. The ratio [^{14}C]UDPglucuronic acid/UDPhexose in cultures fed glucose or galactose was invariably low (0.15–0.2) regardless of the presence or absence of contact inhibition. 20 hr of hexose starvation invariably changed this ratio by a factor of 10 or more, due to accumulation of UDPglucuronic acid. This result was also observed in cultures transformed with polyoma virus. The presence of 3-*O*-methylglucose in the growth medium did not alter the typical "sugar starvation pattern" (i.e., the UDPglucuronic acid/UDPhexose ratio averaged 1.7).

Enhancement of galactose uptake by hexose starvation was very pronounced in NIL cultures that were close to contact inhibition, but was not a prominent feature in the polyoma-transformed cultures. The transformed cells grown on glucose or galactose growth medium showed the usual enhanced rate of uptake of galactose as compared with nontransformed near-confluent cultures that had been fed hexose. The polyoma-induced enhancement showed none of the features characteristic of hexose-starved cells.

Enhancement of hexose uptake in cell cultures seems to be a function of several factors, an important one being transformation of cells by tumor viruses (1–4). The rate of uptake is, however, markedly stimulated by a previous deprivation of glucose from the growth medium (5). Thus, the rate of uptake of glucose or 2-deoxyglucose was enhanced 15- to 20-fold if the growth medium of chick fibroblasts did not contain any glucose (5). Likewise, in untransformed mouse-embryo fibroblasts the initial rates of uptake of glucose and its analogues were about 10-times higher if the growth medium contained D-xylose instead of glucose (6).

Galactose uptake was also enhanced in chick fibroblasts under conditions of previous hexose starvation; addition of

glucose or galactose to the growth medium prevented enhancement of galactose uptake (5).

In a previous article (4) we pointed out that the large enhancement factor observed as a result of glucose starvation posed several problems. Is the supposed transformation-induced enhancement of hexose entry [or of facilitated exchange (7, 8)] merely a result of the excessive glucose consumption that is so characteristic of tumor cells?

We tended to dismiss the latter argument as a result of some of our own studies on galactose uptake into hamster cells (4). The rate of uptake of galactose was 2.5- to 3-fold higher in polyoma-transformed hamster cells compared to nontransformed cells, regardless of the source of hexose during growth (glucose as a fast catabolite, galactose as a slow catabolite). A careful examination of galactose metabolism in hamster cells largely supported this notion. We have found, however, some differences in consumption of galactose by hamster NIL cells transformed with polyoma virus (NILpy) compared to NIL cells, indicating a significantly higher consumption by the former compared to the latter. Hence, we decided to do a more direct and systematic study of the effect of prior hexose starvation of hamster cells (NIL as well as NILpy cells) on the rate of galactose uptake and to compare the types of accumulation products formed under various conditions of enhancement. This study was more rewarding than anticipated because the radioactive accumulation products, stemming from [^{14}C]galactose, were strikingly different even after short incubation times, depending on the various conditions (hexose starvation, alkaline pH, polyoma transformation) to which the hamster-cell cultures had been subjected.

METHODS

Cells were grown in 75-cm² or in 25-cm² Falcon bottles. 24 hr before the uptake test, cells were given MEM-ES in which glucose (1 mg/ml) was added, or replaced by galactose (1 mg/ml), or completely omitted. The growth medium contained 10% dialyzed fetal-calf serum (Gibco). The cells were untransformed, as well as transformed, hamster cells, NIL and NILpy (see ref. 4). In most cases the NIL cultures were close to or at the stage of contact inhibition. However, sparse NIL cultures far from contact inhibition were also used. The NIL cultures were checked by the agar selection method (9) (courtesy of Dr. Hakomori and Miss Maureen Thornton).

After 20–24 hr in growth medium at 37°, the medium was removed and cells were washed three times with phosphate-buffered saline (pH 7.4) at room temperature. [$U\text{-}^{14}\text{C}$]Galactose was added in phosphate-buffered saline plus galactose corresponding to 1 $\mu\text{Ci/ml}$ and a final concentration of 16.5 μM galactose. Uptake tests were done in the flasks for 20 min

Abbreviations: NILpy, hamster NIL cells transformed with polyoma virus; Gal-1-P α -galactose-1-phosphate; UDPHex, uridinediphospho hexose; UDPGlcUA, uridinediphospho glucuronic acid; MEM-ES, see Assoc. Biomedical Systems, Inc., p. 8.

TABLE 1. [^{14}C]Galactose uptake over 20 min at 22° in NIL cultures

	Grown with glucose		Grown with galactose		Grown without hexose	
	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
No. of cells per $\text{cm}^2 \times 10^{-5}$	0.82	0.47	0.80	0.27	0.89	0.23
% Distribution of ^{14}C after chromatography						
UDPGlcUA	4.2	7.1	3.6	5.1	46.2	50.0
UDPHex	57.2	42.5	34.7	34.1	11.1	10.2
Gal-1-P	8.4	9.6	10.2	31.4	11.8	20.8
Gal	26.5	36.2	46.7	23.9	27.9	13.6
"Lactate"	3.5	4.6	4.6	5.7	2.8	13.4
cpm per mg of protein (20 min)	25,725	71,258	26,134	141,586	79,410	182,705

a, Contact inhibited; *b*, sparse cultures.

at room temperature (22°); in some cases 10-min incubations were used. In a few cases a 20-min incubation at 37° was performed. After incubation the medium was removed from the flasks and the cells were washed three times with ice-cold phosphate-buffered saline. The cells were then extracted with warm 70% ethanol while still in the flasks. Protein was dissolved in 0.1 N NaOH and determined by the procedure of Lowry *et al.* (10).

Since the counts in the soluble fraction constituted more than 95% of the counts taken up, the insoluble protein fraction was not routinely counted. Chromatography was done as described (11); 1 mg of protein corresponds to 2.88×10^6 NIL cells (4).

RESULTS

Under the usual culture conditions in galactose growth medium, NIL cultures use only between 8 and 18% of the total amount of galactose in the growth bottle, whereas, NILpy cultures use between 25 and 50% of the total galactose. Hence, the transformed hamster cells do use more galactose over a 24-hr period than untransformed cells. Does this difference in consumption and in amounts in the medium affect the rate of uptake of galactose?

In order to examine this question, we studied the uptake of [^{14}C]galactose in NIL and NILpy cultures starved for hexose over 24 hr. Also, the types of ^{14}C -labeled products that accumulate from galactose during a 10- or 20-min incubation were recorded. Tables 1 and 2 illustrate several characteristic patterns that were disclosed after paper chromatography.

All cultures starved of hexose whether they be sparse or contact-inhibited NIL cultures or NILpy cultures show a pronounced slow-moving ^{14}C peak that is barely detectable in the hexose-fed cultures. We call this slow-moving peak the "UDPGlcUA peak" since it stems from [^{14}C]galactose (see ref. 11) and also to indicate that several of its chemical properties correspond to those of UDPGlcUA.

Enhancement of uptake of [^{14}C]galactose due to hexose starvation is seen only in the more or less contact-inhibited cultures. However, the correlation between hexose starvation and the appearance of the UDPGlcUA peak at the expense of the UDPHex (UDPHex) peak is perhaps most noteworthy. Table 3 summarizes UDPGlcUA accumulation in hexose-starved and hexose-fed cultures, expressed as the ratio of [^{14}C]UDPGlcUA/UDPHex. This census includes NIL cultures more or less contact-inhibited and sparse as well, but not NILpy cultures, although in NILpy cells this ratio was likewise increased by a factor of about 10 after hexose starvation. The marked increase in the ratio was also observed in some NIL samples incubated at 37° for 20 min.

The ratio UDPGlcUA/UDPHex, which is about 0.1 in fed NIL cells, rises 40- to 50-fold (ratios between 4 and 5) in starved cells. Cultures that have received 3-*O*-methylglucose, a sugar that is not phosphorylated by mammalian cells (12), show UDPGlcUA/UDPHex ratios higher than one (see Table 3), although the rise in the number of counts taken up was only 1.5- to 2-fold over that of hexose-fed cultures.

Some of the [^{14}C]UDPGlcUA peaks from hexose-starved cells observed after paper chromatography were eluted. An

TABLE 2. [^{14}C]Galactose uptake over 20 min at 22° in NILpy cultures

	Grown with glucose		Grown with galactose			Grown without hexose	
	1	2	1	2	3	1	2
Sample no.	1	2	1	2	3	1	2
No. of cells per $\text{cm}^2 \times 10^{-5}$	1.98	1.92	1.91	2.20	1.89	1.67	1.18
% Distribution on ^{14}C after chromatography							
UDPGlcUA	4.3	3.6	3.0	4.3	5.8	21.7	30.6
UDPHex	59.3	61.9	64.8	74.4	76.1	31.2	50.2
Gal-1-P	9.0	8.0	6.0	5.6	5.8	34.5	19.5
Gal	18.3	17.0	12.5	10.0	8.0	7.7	15.7
"Lactate"	9.0	9.4	14.3	5.7	4.2	4.7	5.5
cpm per mg of protein (20 min)	34,205	40,602	68,282	48,926	49,350	60,972	69,129

FIG. 1. Scanning from a polyethyleneimine thin-layer chromatogram of a Darco eluate stemming from a [^{14}C]UDPGlcUA peak of a paper chromatogram from hexose-starved cells incubated with ^{14}C -labeled galactose. The concentrated Darco eluate was chromatographed by the method described in *text*.

aliquot was counted, then adsorbed on Darco, which was subsequently washed with water and eluted with 50% ethanol, pH about 8. A fraction of the concentrated eluate was cochromatographed on polyethyleneimine thin-layer plates in Randerath's LiCl-acetic acid mixture M (13), with 20 nmol of authentic UDPGlcUA. It appears from Fig. 1 that the radioactive peak coincides with the appearance of the UV-quenching spot of UDPGlcUA. Analysis of the labeled UDPGlcUA by a specific UDPglucuronate lyase (14) with our chromatographic technique (11) showed full conversion to UDPxylose.

DISCUSSION

The major facts that emerge from the present study are the following. NIL hamster cultures close to contact inhibition show greatly enhanced uptake of ^{14}C -labeled galactose if they have previously been subjected to hexose starvation. The most characteristic feature of this hexose-starvation profile is the striking amounts of UDPGlcUA that accumulate, mainly at the expense of UDPHex. In NIL cultures that are exempt from contact inhibition, such as very sparse cultures, alkaline cultures (14), or polyoma-transformed cultures, the rate of uptake of [^{14}C]galactose was high even in hexose-fed cultures. Only moderate increases in the rate of uptake of [^{14}C]galactose were observed in the hexose-starved sparse NIL cultures. However, sparse cultures subjected to hexose starvation in the growth medium also showed the typical sharp rise in ^{14}C -labeled UDPGlcUA. The events that lead to the formation of ^{14}C -labeled UDPGlcUA are probably rather involved. They may just as well be elicited from metabolic events as from the variations in transport through membrane. In any event, the accumulation of UDPGlcUA is not correlated with the enhanced rate of uptake of galactose in any simple way.

The metabolic aspect of the rapid accumulation of [^{14}C]UDPGlcUA from administered [^{14}C]galactose is perhaps best illustrated by the following experiment. The sugar 3-O-methylglucose, which is able to participate in facilitated exchange (7, 8) but is not phosphorylated (12), when added to the growth medium instead of glucose or galactose, did not change the "starvation pattern," i.e., the UDPGlcUA/UDPHex ratio remained high (see Table 3).

Amos and coworkers report that addition of insulin to chick fibroblasts in a hexose-free medium within a few hours permits a rapid enhancement of glucose uptake (15). It would be important to try the effect of insulin in the same fashion

TABLE 3. UDPGlcUA accumulation in both sparse and contact-inhibited NIL cultures

Growth medium	UDPGlcUA	
	UDPHex (average $\pm$ SD)	No. of samples
+ Glucose	0.19 $\pm$ 0.11	8
+ Galactose	0.15 $\pm$ 0.06	13
No Hexose	2.60 $\pm$ 1.26	14
+ 3-O-MeGlucose	1.74	3(1.99, 2.20, 1.03)

in NIL cultures and see the response in terms of conversion of [^{14}C]galactose to UDPGlcUA.

It will also become necessary to include enzyme studies on broken cells in order to see if the activity of certain enzymes, like, for instance, UDPG dehydrogenase, is greatly increased in cultures previously starved of hexose. However, studies on enzyme activities in broken cells can be misleading unless the relative concentrations of substrate and feedback inhibitors that are apt to be present in the intact cells are simulated as closely as possible in the *in vitro* metabolic studies. It is, of course, difficult to fulfill such prerequisites. As mentioned in a previous article (4) we have been able to demonstrate a striking example of regulatory inhibition in intact NIL and NILpy cells, namely the choke of epimerase activity. The epimerase choke is promoted to a great extent by NADH present in the cell (11). The latter is again a function of factors like ATP concentrations and, especially, the ratio ATP to ADP (16). Galactokinase concentrations may vary considerably in intact cells although it may not be evident from studies on broken-cell preparations (4).

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- Hatanaka, M. & Gilden, R. V. (1970) *J. Nat. Cancer Inst.* **45**, 87-89.
- Martin, G. S., Venuta, S., Weber, M. & Rubin, H. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2739-2741.
- Isselbacher, K. J. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 585-589.
- Kalckar, H., Ullrey, D., Kijimoto, S. & Hakomori, S. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 839-843.
- Martineau, R., Kohlbacher, M., Shan, S. N. & Amos, H. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3407-3411.
- Hatanaka, M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1364-1367.
- Venuta, S. & Rubin, H. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 653-657.
- Weber, M. (1973) *J. Biol. Chem.* **248**, 2978-2983.
- Macpherson, I. & Montagnier, L. (1964) *Virology* **23**, 291-294.
- Lowry, O. H., Rosebrough, N. J., Farr, L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275.
- Robinson, E. A., Kalckar, H. M., Troedsson, H. & Sanford, K. (1966) *J. Biol. Chem.* **241**, 2737-2745.
- Narahara, H. T. & Pinar, O. (1963) *J. Biol. Chem.* **238**, 40-49.
- Randerath, K. & Randerath, E. R. (1967) in *Methods of Enzymology*, eds. Grossman & Moldava (Academic Press, New York), Vol. XIIA, pp. 323-347.
- Ankel, H. & Feingold, D. S. (1966) *Biochem.* **5**, 182-189.
- Shaw, S. N. & Amos, H. (1973) *Biochem. Biophys. Res. Commun.*, in press.
- Chance, B. (1957) in *Enzymes: Units of Biological Structure and Function* (Academic Press, New York), pp. 447-463, Fig. 14.