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Increased Uptake of Amino Acids and 2-Deoxy-D-Glucose by Virus-Transformed Cells in Culture

(lectins/mouse/hamster/SV40/polyoma)

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ABSTRACT **Transformed and nontransformed cells in tissue culture differ in their rate of uptake of certain nutrients, as determined by a polyester-coverslip technique. A 2.5- to 3.5-fold increased rate of uptake of α-aminoisobutyric acid, cycloleucine, and 2-deoxy-D-glucose was observed with polyoma virus-transformed baby hamster kidney (BHK) 21 cells and simian virus 40 (SV40)-transformed BALB/3T3 (mouse fibroblast) cells, compared to their nontransformed counterparts. Kinetic analysis suggested that the increased uptake by cells transformed with virus was associated with a 3-fold greater V_{max}, with no detectable changes in apparent K_m. Limited studies also revealed increased initial rates of uptake by murine sarcoma virus-transformed rat liver cells, as compared to the parental line. Exposure of cells to concanavalin A and wheat-germ agglutinin led to significant reductions in amino-acid uptake by both transformed and nontransformed cells; however, transformed cells showed a greater decrease in uptake after exposure to wheat-germ agglutinin. Increased initial rates of uptake of certain amino acids and sugars may be a feature common to transformed cells, compared to their parental control.**

Several investigators have examined the possibility that uptake or transport of nutrients by cells is altered in association with malignancy. Eagle, Piez, and Levy (1) found no reproducible differences in the ability of several cell lines derived from normal and malignant tissues to accumulate amino acids. Hare (2) also found no definite changes in the characteristics of phenylalanine transport by virus-transformed hamster cells. However, in a study of 3T3, and polyoma virus-transformed 3T3, cells, Foster and Pardee (3) observed a greater rate of uptake and V_{max} for α-aminoisobutyric acid and cycloleucine by the transformed cells.

Recently, other investigators have observed striking changes in the initial rates of uptake of certain hexoses, especially 2-deoxy-D-glucose, by transformed cells (4–9). Hatanaka *et al.* found 6- to 15-fold increases in the rate of uptake of this sugar by mouse (5, 7) and rat cells (4) transformed by murine sarcoma virus, and in chicken cells transformed by Rous sarcoma virus (6). This change was associated with a markedly lower K_m for sugar transport. Since these workers found that cells infected with leukemia virus do not show this change, they considered changes in the value of K_m to be related to the expression of specific sarcoma virus genes. Hatanaka *et al.* (6) did not observe any difference in the rate of transport of L-leucine.

I examined in greater detail the kinetics of uptake by two normal cell lines and their DNA virus-transformed derivatives; namely, BHK 21 and its polyoma virus-transformed counterpart, and BALB/3T3 and SV40-transformed BALB/3T3 cells. The effect on uptake by the lectins concanavalin A (Con A) and wheat-germ agglutinin (WGA), which react with the plasma membrane, was also studied. The technique of Foster and Pardee (3) was modified to study transport with cells attached to plastic coverslips. Measurements of uptake, radioactivity, and protein were all made directly on the coverslips. Significantly greater initial rates of uptake (2.5- to 3.5-fold) occurred with the polyoma- and SV40-transformed cells. These changes were associated with an increase in V_{max}, but with no change in K_m.

MATERIALS AND METHODS

Cells and Culture Methods. Most experiments were done with BHK 21 (a hamster cell line; Cl3 clone) (10) and a polyoma-transformed derivative (PyJ) (10) or BALB/3T3 and SV40-transformed BALB/3T3 cells. Coverslips (15 × 17 mm) were cut from "Melinex" (Type O, 50 μm thick, Imperial Chemical Industries, Garden City, Herts, England). They were sterilized in 90% ethanol, and placed in special compartmentalized plastic dishes ("Replidishes", Dyos Plastics, Surbiton, Surrey, England).

Cells were cultured in Dulbecco's modification of Eagle's medium containing 10% fetal-calf serum, and were seeded at initial counts of 2–2.5 × 10^4 cells per compartment. Studies were usually performed after 3 days, so that relatively dense, but nonconfluent, cultures were obtained. For studies in G1 phase, cells were incubated for 3 days in 0.5% fetal-calf serum (11).

Uptake Methods. To study amino-acid uptake, cells were washed twice with phosphate-buffered saline (PBS, pH 7.2) at 37°, and were then incubated with 1 ml of Earle's saline for 45 min in a 37° water bath (room air) to reduce the intracellular pool of amino acids (3). Since Earle's saline contains glucose, cells were first incubated 45 min in PBS for hexose-uptake experiments; however, such incubation gave a slightly lower basal level of amino-acid uptake. After such incubation, labeled substrate (2.0 or 0.02 mM; 0.167 μCi/ml) was injected into each Replidish compartment. Except where indicated, uptake studies were for 10 min. The medium was then aspirated and the coverslips were removed and rinsed

Abbreviations: PBS, phosphate-buffered saline (pH 7.20), Con A, concanavalin A; WGA, wheat-germ agglutinin; Py, polyoma virus.

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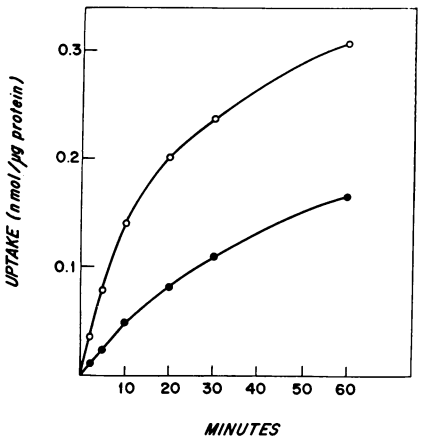

FIG. 1. Uptake of α-aminoisobutyrate by BHK 21 and PyJ cells. At the time of study, cells were nonconfluent; BHK 21 cells had a mean count of 8.5×10^4 per coverslip and 42 μg of protein; PyJ cells had a mean count of 1.2×10^5 per coverslip and 58 μg of protein. Cells were washed with PBS and incubated with 1 ml of Earle's saline for 45 min. α-Aminoisobutyrate was then added to give a concentration of 2.0 mM (0.167 μCi/ml). Each point represents the mean of triplicate or quadruplicate determinations. ●—●, BHK 21 cells; O–O, PyJ cells.

rapidly (10 sec) by dipping them serially through PBS at 37°. After they were dried, the coverslips were transferred to vials containing Bray's solution (12) for liquid scintillation assay. Each experimental point represented the mean uptake on 3 or 4 coverslips. Protein measurements (13) were made in duplicate on the cells attached to the coverslips. I confirmed that a fairly good linear relationship existed between cell number and protein estimation for cell counts less than 8×10^5 per coverslips (3).

Isotopes and Chemicals. [^{14}C] α-aminoisobutyric acid (58 g/Ci) was obtained from the Radiochemical Center, Amersham. [Carboxyl-^{14}C]-1-aminocyclopentane-1-carboxylic acid (cycloleucine) and [1-^{14}C]-2-deoxy-D-glucose were obtained from New England Nuclear Corp. at specific activities of 2 and 50 μCi/mmol, respectively. L-[^{14}C]Arginine (220 mCi/mmol), and L-[^{14}C]glutamic acid (180 mCi/mmol) were obtained from New England Nuclear Corp. Con A, twice crystallized, was obtained from Sigma Chemical Co. Partially purified WGA was a gift of Dr. John Ewart, Flour Milling and Baking Research Ass., Chorleywood, Rickmansworth, England.

TABLE 1. *Uptake of amino acids and 2-deoxy-D-glucose by BHK 21 and PyJ cells*

Substrate	Concentration mM	No. of exps.	Uptake BHK 21 (A) nmol per mg of protein per min	Uptake PyJ (B) nmol per mg of protein per min	Ratio B/A
α-Aminoisobutyrate	2.0	12	5.25	14.13	2.7
	2.02	8	0.19	0.63	3.3
Cycloleucine	2.0	12	4.84	14.31	3.0
	0.02	5	0.15	0.52	3.5
Arginine	0.02	2	0.043	0.22	5.1
2-Deoxy-D-glucose	0.02	3	0.014	0.049	3.5

Studies were performed on nonconfluent cells. After washing with PBS, cells were incubated for 45 min with 1 ml of Earle's saline (or with PBS in studies with deoxyglucose). Isotope (0.167 μCi) was added to give the concentrations indicated. Incubations were at 37° for 10 min. Each experiment was carried out in triplicate or quadruplicate.

TABLE 2. *Kinetic constants for the uptake of α-aminoisobutyrate and cycloleucine*

Cell line	Substrate: α-Aminoisobutyrate K_m	α-Aminoisobutyrate V_{max}	Cycloleucine K_m	Cycloleucine V_{max}
BHK 21	1.4	110	1.0	130
PyJ	0.9	300	0.9	320
3T3	1.5	65	0.8	55
SV40-transformed 3T3	1.3	170	1.0	150

Kinetic values were calculated from Lineweaver–Burke plots. K_m is expressed as mM/liter; V_{max} as picomol per μg of protein per 10-min. Assays and incubations were as in Table 1; substrate concentrations ranged from 20 to 0.01 mM.

RESULTS

Uptake of α-aminoisobutyrate and cycloleucine by BHK 21 and PyJ cells

Fig. 1 shows typical rates of uptake of α-aminoisobutyrate by nonconfluent BHK 21 cells and the corresponding polyoma-transformed cell line, PyJ. Uptake of both α-aminoisobutyrate and cycloleucine was relatively linear for 20–30 min with both cell lines. At all times, PyJ cells had a consistently 2.5- to 3.5-fold greater rate of amino-acid uptake than did the nontransformed cells.

To standardize uptake studies and to compare initial rates, most incubations were for 10 min, and results were expressed as nanomoles of amino acid taken up per mg of cell protein per min. α-Aminoisobutyrate and cycloleucine (at 2 and 0.02 mM) were taken up at faster rates by PyJ cells (Table 1). The ratio of uptake by nonconfluent PyJ compared to BHK 21 cells ranged between 2.7 and 3.5. BHK 21 cells cultured for 3 days in 0.5% fetal-calf serum showed no significant differences in initial rates of uptake as compared to BHK 21 cells in the logarithmic phase of growth. It was not possible to make accurate uptake measurements with confluent cultures, since with cell numbers exceeding 1.5×10^6 per coverslip both BHK 21 and PyJ cells tended to pile up and detach during the washing and incubation procedures.

Because Hatanaka *et al.* (4–8) reported increased rates of deoxyglucose transport by chick and mouse cells transformed with murine and Rous sarcoma viruses, but not with SV40-transformed 3T3 cells, I examined deoxyglucose uptake. Cells were incubated with PSB instead of Earle's saline solution (see *Methods*). I observed a 2.5- to 3.5-fold greater initial rate of uptake of deoxyglucose (at 0.02–2.0 mM) by PyJ than by BHK cells.

The kinetics of α-aminoisobutyrate and cycloleucine uptake were studied with both transformed and nontransformed

TABLE 3. *Uptake of amino acids and 2-deoxy-D-glucose by 3T3 and SV40-transformed 3T3 cells*

Substrate	Concentration mM	No. of exps.	Uptake 3T3 (A) nmol per mg of protein per min	Uptake SV3T3 (B) nmol per mg of protein per min	Ratio B/A
α-Aminoisobutyrate	2.0	4	3.12	7.75	2.5
	0.02	4	0.07	0.22	3.1
Arginine	2.0	2	1.82	4.55	2.5
	0.02	2	0.037	0.097	2.6
2-Deoxy-D-glucose	2.0	2	1.64	3.77	2.3
	0.02	2	0.035	0.082	2.4

Uptake experiments were done on nonconfluent BALB-3T3 and SV40-transformed BALB/3T3 cells after 3 days of culture in medium containing 10% fetal-calf serum. Incubations, isotope concentrations, and assays were as in Table 1. Each experiment was done with triplicate or quadruplicate coverslips.

cell lines. There was a consistently greater V_{max} for α-aminoisobutyrate and cycloleucine with PyJ cells compared to BHK 21, and by SV40-transformed 3T3 cells compared to 3T3 cells (Table 2). However, in contrast to the decreased K_m reported for murine and Rous sarcoma virus-infected cells (4–8), no change in apparent K_m for α-aminoisobutyrate or cycloleucine was observed in these experiments. Although not shown in Table 2, the K_m for deoxyglucose was similar for PyJ and BHK 21 cells, namely, about 1.2 mM.

Studies with 3T3, SV40-transformed 3T3, and other transformed cells

Experiments analogous to those of Foster and Pardee (3) were done with BALB/3T3 and SV40-transformed BALB/3T3 cells. Initial rates of uptake of α-aminoisobutyrate and cycloleucine by SV40-transformed cells were 2.5- to 3.1-times greater than by 3T3 cells (Table 3). The changes in V_{max} (Table 2) were of comparable magnitude. There was a 2.3- to 2.4-fold greater rate of uptake of deoxyglucose by the SV40-transformed cells (at both 0.02 and 2.0 mM).

Comparable results were obtained with an epithelial cell line of rat liver cells, and the corresponding cell line transformed by murine sarcoma virus†. Transformed cells showed a 3.6- to 3.7-fold greater rate of α-aminoisobutyrate uptake and a 4-fold increase in their deoxyglucose uptake (Table 4).

Effect of lectins on uptake by normal and transformed cell lines

The effect of various agglutinins on uptake was examined, since several lectins cause preferential agglutination of transformed cells (13–17). When PyJ cells were incubated with 100 μg/ml of Con A or WGA, cycloleucine uptake was inhibited (75 and 47%, respectively; Fig. 2). This effect could be prevented or modified by the use of the appropriate haptens, which bind preferentially to the lectin and presumably displace it from the cell surface. Thus, when cells were incubated with Con A together with 0.04 M α-methylglucoside, no inhibition of cycloleucine uptake was detected. Furthermore, the inhibition produced by Con A could be reversed by washing the cells with PBS and then incubating them in the presence of (0.04 M) α-methylglucoside. A similar effect could be produced by simultaneous or subsequent incubation of the cells with WCA and *N*-acetylglucosamine (Fig. 2). Results with α-aminoisobutyrate were comparable to those with cycloleucine.

† These cells were kindly provided by Dr. I. B. Weinstein, Institute of Cancer Research, Columbia University.

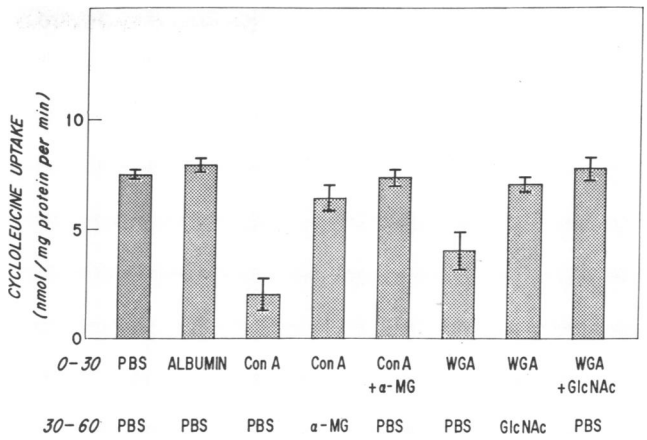

FIG. 2. Effect of WGA and Con A on [^{14}C]cycloleucine uptake by PyJ cells. Transport studies were done with PyJ cells seeded at 2.5×10^4 cells per coverslip and cultured for 3 days in 10% fetal-calf serum. The average number of cells was about 10^5 cells per coverslip (52–58 μg of protein). Cells were incubated from 0 to 30 min with either PBS or PBS containing 100 μg/ml of albumin, Con A, or WGA as shown. The medium was then removed and the cells were incubated for an additional 30 min (as shown) with either PBS or PBS containing 0.04 M α-methylglucoside or *N*-acetylglucosamine. In two sets of experiments, initial incubation was with Con A plus α-methylglucoside WGA plus *N*-acetylglucosamine (*GlcNAc*). After 60 min, [^{14}C]cycloleucine was added (2 mM, 0.167 μCi/ml) and uptake was measured after 10 min. Results represent the mean and range from five sets of experiments. *α-MG*, α-methylglucosides.

Table 5 summarizes results obtained with several concentrations of Con A and WGA on transformed and nontransformed BHK and 3T3 cells. At all concentrations tested, Con A decreased α-aminoisobutyrate uptake by *both* the transformed and nontransformed cells, and to about the same extent. However, a differential effect was noted with WGA. At 50 and 100 μg/ml, WGA inhibited uptake by PyJ cells and produced a lesser effect on uptake by BHK 21 cells. However,

TABLE 4. *Uptake by normal and transformed rat liver (RL and MSV-RL)**

Substrate	Concentration mM	Uptake RL (A) mmol per mg of protein per min	Uptake MSV-RL (B) mmol per mg of protein per min	Ratio B/A
α-Aminoisobutyrate	2.0	12.2	45.6	3.7
	0.02	0.45	1.62	3.6
2-Deoxy-D-glucose	0.02	0.11	0.45	4.0

* Both RL and MSV-RL cells were kindly provided by Dr. I. B. Weinstein, Institute for Cancer Research, Columbia University.

TABLE 5. *Effect of lectins on uptake of 2 mM α-aminoisobutyrate*

Addition	Concentration μg/ml	BHK 21 Uptake nanomol per mg of protein per min	BHK 21 Change % of control	PyJ Uptake nanomol per mg of protein per min	PyJ Change % of control	3T3 Uptake nanomol per mg of protein per min	3T3 Change % of control	SV-3T3 Uptake nanomol per mg of protein per min	SV-3T3 Change % of control
None	0	5.23	100	14.14	100	3.1	100	7.75	100
WGA	50	5.31	82	6.82	48	2.96	95	5.41	70
	100	3.36	63	5.13	36	2.90	93	4.48	58
Con A	50	2.61	50	8.35	59	2.28	73	3.1	40
	100	1.36	26	7.20	51	1.53	49	3.1	25
	300	1.10	21	3.39	24	—	—	—	—

the effect of WGA on uptake by 3T3 and SV40-transformed 3T3 cells was more clear-cut (Table 5). Thus, at 50 and 100 μg of WGA/ml, α-aminoisobutyrate uptake by SV40-transformed 3T3 cells was 70 and 58% of the control value; however, there was little or no effect of WGA on uptake by 3T3 cells.

DISCUSSION

These results show that differences in the rates of uptake of certain amino acids and sugars can be detected between transformed and nontransformed cells. 2.5- to 3.5-fold greater initial rates of uptake and increases in V_{max} were consistently found with nonconfluent polyoma-transformed BHK 21 cells (PyJ), SV40-transformed BALB/3T3 cells, and murine sarcoma virus-transformed rat liver cells than with the respective parental line. Kinetic studies failed to disclose any significant differences in the apparent K_m for α-aminoisobutyrate, cycloleucine, and deoxyglucose by the transformed as compared with the nontransformed cells.

The present results are comparable to those of Foster and Pardee (3) for the uptake of α-aminoisobutyrate and cycloleucine by 3T3 and polyoma-transformed 3T3 cells. They observed a 2- to 3-fold greater uptake and increase in V_{max} of α-aminoisobutyrate and cycloleucine by Py 3T3 cells; they also found no differences in K_m or changes in the rate of uptake of glutamic acid and arginine. In contrast to their findings, we observed that the rates of uptake of arginine (Table 3) and glutamic acid were similar to those seen with α-aminoisobutyrate and cycloleucine.

Recently, others (4–9), have demonstrated changes in hexose transport by cells infected with either murine or Rous sarcoma virus. Hatanaka *et al.* (4–8) reported 6- to 15-fold increases in deoxyglucose uptake by the transformed cells, together with a decrease in apparent K_m (about 10-fold) for hexoses by the sarcoma virus-transformed cells. In their experiments, these changes in apparent K_m were not observed with SV40-transformed and mouse leukemia virus-infected cells (5). Martin *et al.* (9) have shown that deoxyglucose uptake by a temperature-sensitive mutant of Rous sarcoma virus is greater at the permissive temperature.

In contrast to the present results and those of Foster and Pardee (3), Hatanaka *et al.* (6) found no difference in the uptake of L-leucine by Rous sarcoma virus-transformed cells. This discrepancy may be related to the conditions under which the uptake experiments were performed. I confirmed that uptake of amino acids was much diminished with cells whose intracellular amino-acid pool had not been reduced by prior incubation in the absence of amino acids (3); in fact, prior incubation was necessary to demonstrate differences in amino-acid uptake between transformed and nontransformed cells.

Although Lineweaver–Burk plots permit calculations of apparent K_m and V_{max}, one cannot conclude that the uptake process itself conforms to classic Michaelis-Menten kinetics. However, V_{max} and K_m values may be useful for comparison of uptake characteristics. Changes in V_{max} reflect the number of sites involved in the carrier-mediated process, while differences in K_m reflect qualitative alterations of these sites. Thus, changes in V_{max} for amino-acid and deoxyglucose uptake may reflect alterations common to many transformed cells due to an increase in the *number* of membrane sites involved in uptake. On the other hand, decreases in K_m (4–8) may be more specific and reflect changes in the *nature* of the uptake sites. Some of these issues may be resolved by uptake studies with plasma-membrane vesicles prepared from transformed and nontransformed cells.

Since the changes in amino-acid and sugar uptake may involve alterations in cell surfaces (7), the effects of lectins on the uptake process was of interest. WGA and Con A selectively agglutinate cells transformed by oncogenic viruses. This agglutination is inhibited by carbohydrate haptens believed to be sterically similar to the cell-surface lectin-binding sites (14–18). Agglutination by WGA is inhibited by *N*-acetylglucosamine and that by Con A is inhibited by α-methylglucoside. As shown in Table 5 and Fig. 2, concentrations of Con A that produced preferential agglutination of cells transformed by polyoma and SV40 decreased amino-acid uptake by the transformed cells. However, uptake by nontransformed BHK 21 and 3T3 cells was also decreased. In both instances the effect was reversible or prevented by the subsequent addition or presence of α-methylglucoside.

It is of interest that Con A produced selective agglutination of transformed cells, but decreased amino-acid uptake by *both* nontransformed and transformed cells. There appears to be no positive correlation between the number of cell-surface lectin-binding sites and the agglutination effects of Con A and WGA (19, 20). The elucidation of the mechanism of action of Con A is further complicated by the fact (21) that commercial Con A contains several molecular species and several subunit fragments. Agglutination could be caused by

one of these species, while the effects on transport might involve the action of several fragments or subunits.

While there also appears to be no difference in the number of WGA-binding sites between normal and transformed cells (19, 20), preferential decrease in amino-acid uptake occurred when WGA was added to transformed cells (Table 5). This was especially the case with SV40-transformed 3T3 and 3T3 cells. The reason for this differential effect between Con A and WGA on uptake is not clear. Conceivably, different surface receptor sites are involved with WGA and Con A binding to topologically distinct areas on the cell surface (20, 22). The use of nonagglutinating monomeric subunits of Con A (23) may clarify the lectin effects on transport.

The important question of whether the changes in uptake can be used as a specific measure of transformation requires further study. However, Martin *et al.* have suggested that under appropriate conditions the increased nutrient uptake by transformed cells is specific and is not dependent on increased rates of cell division (9).

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