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Differential Uptake of 2-Fluoro-2-deoxyglucose by Normal and Transformed Chicken and Mouse Fibroblasts as a Function of Glucose Concentration*

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Abstract—*Recently,* [^{18}F]-2-*fluorodeoxyglucose, a positron emitter, has found an important application in certain metabolic studies using external scanning techniques. We have studied* [^{14}C]-2-*fluorodeoxyglucose,* [^{3}H]-2-*deoxyglucose and* [^{14}C]-D-*glucose uptake in normal and transformed chicken and mouse fibroblasts to determine whether such external scanning techniques might be applicable to detect* in vivo *tumoral localisations. In this* in vitro *model, with glucose concentrations in the culture medium below that of normal animal blood, transformed cells showed a higher uptake of all three hexose tracers than did normal cells. However, at physiological glucose concentrations there was no differential uptake of these hexoses. Therefore our results suggest that* [^{18}F]-*fluorodeoxyglucose would be of no help in the* in vivo *detection of tumour localisations by external positron scanning unless* in vitro *and* in vivo *cell behaviour differs considerably.*

INTRODUCTION

CELLS transformed *in vitro* by viruses [1–3], or by chemical carcinogens [4] transport hexoses at a higher rate than their normal cell counterparts. Two mechanisms appear to be involved in the transport of hexoses across the cell membrane: a saturable carrier-mediated process called facilitated diffusion, and a non-saturable process including simple diffusion [5]. Alterations of both modes of transport in transformed cells have been claimed, but there is still controversy about the precise significance of some results [2, 6–9]. For example, hexose retention by phosphorylation inside the cells is considered by Romano and Colby [10] to be the cause of the high hexose uptake by transformed cells, without direct change of their transport ability.

One of the most used hexose analogues is 2-deoxyglucose (2dg), which is trapped by phosphorylation inside the cells and not further metabolized [5, 11]. The availability of the positron-emitting [^{18}F]-2-fluorodeoxyglucose (Fdg) and of positron-computed tomography [12] might provide a means to detect tumoral tissues *in vivo* by external scanning if tumour cells take up and trap Fdg more efficiently than normal tissues. Indeed, this technique has already permitted a physiological study of the human brain [13] by external scanning.

Therefore we investigated the *in vitro* uptake of Fdg, 2dg and glucose in normal and virus-transformed chicken and mouse fibroblasts with respect to the glucose concentration in the medium. We report here that the differential hexose uptake observed with these 3 hexoses at low external glucose concentrations disappeared when the glucose concentration reached the physiological (for most animal blood) level of 5.5 mM. Our results do not favour the hope that Fdg might help to detect *in vivo* tumours and metastases by external positron scanning.

MATERIALS AND METHODS

Cell viruses and culture medium

Chicken embryo fibroblasts (CEF) were secondary cultures originating from 10-day-old Brown Leghorn C/O embryos. About 3×10^5 cells were seeded in 3.5-cm dishes and infected

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with the Schmidt–Ruppin strain, subgroup A, of Rous sarcoma virus (SR-A RSV), at an m.o.i. of 0.1. When the majority of the cells was morphologically transformed, these cultures, together with non-infected control cultures, were used for hexose uptake determinations. The cell number per dish varied from 1.6 to 2×10^6.

C127 cells belong to a continuous cell line derived from embryo fibroblasts of the mouse RIII strain. Their transformed counterpart, hereafter called C127-Ki, is an isolate from C127 cells transformed with Kirsten sarcoma virus [14]. These cells are also infected with Moloney mouse sarcoma virus as a helper, and produce both viruses. Both cell lines were the gift of Dr. Scolnick. C127 and C127-Ki cells were seeded in 3.5-cm dishes (10^5 cells) and used 2 or 3 days later, when their number was about $1.5–2 \times 10^5$.

The culture medium was a modified Eagle's medium, with a double concentration of vitamins, essential and non-essential amino-acids in Earle's salt solution (glucose concentration: 22 mM).

The medium was supplemented with antibiotics and 5% calf serum. Cultures were incubated at 37°C in an atmosphere of air +5% CO_2. Glucose concentration of culture media was assayed using a UV-Test kit (Boehringer).

Hexose uptake

Hexose uptake was performed as follows at 37°C on parallel cultures of control and transformed cells. Dishes were washed twice with warm Earle's solution containing the same glucose concentration as that to be used for the labelling. The labelling medium consisted of 1 ml of Earle's solution containing 2-deoxy-2-fluoro-D-U-[^{14}C]glucose (0.14 μCi/ml, sp. act. 343 mCi/mmol, New England Nuclear), 2-deoxy-D-1-[^{3}H]glucose (0.25 μCi/ml, sp. act. 19 Ci/mmol, Amersham) or D-U-[^{14}C]glucose (0.1 μCi/ml, sp. act. 260 mCi/mmol, Amersham). The indicated final hexose concentrations were obtained by the appropriate addition of glucose. Labelling was stopped by rapid aspiration of the radioactive solution and twice washing with 2.0 ml of ice cold Earle's solution (glucose concentration 5.5 mM). Then the cells were dissolved in 0.1 N NaOH and aliquots were counted in Bray's solution in a Packard scintillation counter. The number of cells per dish was determined on parallel cultures.

Cytochalasin B (CB) was dissolved in dimethyl-sulfoxide (DMSO) and diluted into the labelling solution so that the cells received simultaneously both the inhibitor and the hexose sugar. Control cultures were given an equal amount of DMSO. The final concentration of DMSO was 0.25% and of CB, 10 μg/ml.

RESULTS

In an initial experiment we checked to what extent the differential uptake of 2dg by normal and transformed cells was dependent on the glucose concentration in the labelling medium. It can be seen in Table 1 that for 10-min labelling periods, the greater uptake by transformed cells over normal cells decreased when the glucose concentration increased, and was completely eliminated at the physiological concentration of 5.5 mM. Basically, the same results were obtained when [^{14}C]-D-glucose and [^{14}C]-Fdg were used as tracers, i.e. the difference of uptake between normal and transformed cells was clear at low hexose concentrations but was no longer observed at an external glucose concentration of 5.5 mM (Table 2). It is noteworthy that at low hexose concentrations this difference in uptake was frequently more marked for [^{14}C]-Fdg than for [^{3}H]-2dg, but not always (see Table 3). Like many culture media, the one used here, prior to labelling, contains a higher concentration of D-glucose (22 mM) than blood to avoid severe glucose depletion after several hours of cell growth. We checked that this high pre-labelling glucose concentration did not significantly modify the subsequent hexose uptake by the cells. Parallel cultures of normal and transformed CEF and of normal and transformed C127 cells were maintained for 24 hr in 5.5 mM D-glucose culture media before measuring the uptake of [^{3}H]-2dg and [^{14}C]-Fdg. The difference of uptake between normal and transformed cells was clear at low hexose concentrations in the labelling medium (380 mM) and no longer observed when the hexose concentration was 5.5 mM in the labelling medium (data not shown).

One may suppose that at high external hexose concentrations the overwhelming uptake by simple diffusion might have obscured a difference of uptake only or mainly due to the facilitated mode of transport. To discriminate between facilitated and simple diffusion we used cytochalasin B, which inhibits the facilitated hexose transport but not the simple diffusion [15, 16]. Detailed results with chicken and mouse cells labelled with [^{3}H]-2dg or [^{14}C]-Fdg are shown in Table 3. At a low hexose concentration in the medium (380 mM) cytochalasin B suppressed the increased uptake of hexoses by transformed cells, showing that only

Table 1. Uptake of [^{3}H]-2-deoxyglucose by normal and transformed chicken or mouse cells as a function of D-*glucose concentration in the labelling medium*

D-glucose concentration in the labelling medium	[^{3}H]-2dg uptake in (pmol per 10^6 cells per 10 min) Chicken cells CEF	SR-A RSV CEF	Ratio transformed: control	Mouse cells C127	C127-Ki	Ratio transformed: control
0		not done		0.479	0.963	2.01
0.1 mM	0.058	0.453	7.81	0.390	0.580	1.48
0.5 mM	0.056	0.240	4.28		not done	
2.0 mM	0.037	0.058	1.56	0.260	0.236	0.91
5.5 mM	0.030	0.032	1.06	0.264	0.198	0.75

Cell cultures were labelled for 10 min with 0.25 μCi per ml of [^{3}H]-2dg (this is equivalent to 13 nM) in Earle's solution and the final hexose concentrations were adjusted by addition of D-glucose. In this and in the following tables, the given values represent the mean of duplicate values; in no single case did the variation between duplicate values exceed 15%.

*Table 2. Comparative uptake of [^{3}H]-2-deoxyglucose, [^{14}C]-*D*-glucose and [^{14}C]-2-fluorodeoxyglucose by normal and transformed chicken and mouse cells as a function of* D-*glucose concentration*

Tracer sugar	Hexose concentration	[^{3}H]-2dg, [^{14}C]-D-glucose and [^{14}C]-Fdg uptake (pmol per 10^6 cells per 10 min) Chicken cells CEF	SR-A RSV CEF	Ratio transformed control	Mouse cells C127	C127-Ki	Ratio transformed control
[^{3}H]-2dg	380 nM	0.123	1.064	8.65	0.431	0.739	1.71
	5.5 mM	0.091	0.071	0.78	0.264	0.198	0.75
[^{14}C]-D-glucose	380 nM	1.779	14.909	8.38	8.939	45.223	5.05
	5.5 mM	0.913	1.071	1.17	5.834	5.734	0.98
[^{14}C]-Fdg	380 nM	2.360	33.828	14.33	17.656	60.944	3.45
	5.5 mM	1.189	0.838	0.70	5.965	6.136	1.02

Cell cultures were labelled for 10 min with 0.25 μCi/ml of [^{3}H]-2dg, 0.1 μCi/ml of [^{14}C]-D-glucose or 0.14 μCi/ml of [^{14}C]-Fdg (13 nM, 380 nM and 380 nM, respectively) in Earle's solution and the final hexose concentrations were adjusted by addition of D-glucose.

Table 3. [^{3}H]-2*dg and* [^{14}C]-*Fdg uptake by normal and transformed cells in presence or absence of cytochalasin B (CB) as a function of hexose concentration in the labelling medium*

Labelling sugar and hexose concentration in the medium	Chicken Cells						Mouse cells					
		CEF			SR-A RSV CEF			C127			C127-Ki	
	Total	CB resistant	CB sensitive	Total	CB resistant	CB sensitive	Total	CB resistant	CB sensitive	Total	CB resistant	CB sensitive
[^{3}H]-2dg												
380 nM (% of total uptake)	0.039	0.017 (44)	0.022 (56)	0.197	0.016 (8)	0.181 (92)	0.084	0.052 (62)	0.032 (38)	0.218	0.058 (27)	0.160 (73)
5.5 mM (% of total uptake)	0.026	0.013 (50)	0.013 (50)	0.019	0.011 (58)	0.008 (42)	0.056	0.039 (70)	0.017 (30)	0.060	0.046 (77)	0.014 (23)
[^{14}C]-Fdg												
380 nM (% of total uptake)	4.305	0.795 (18)	3.510 (82)	23.812	1.047 (4)	22.765 (96)	8.455	2.480 (29)	5.975 (71)	29.503	3.294 (11)	26.209 (89)
5.5 mM (% of total uptake)	1.926	0.544 (28)	1.382 (72)	1.027	0.486 (47)	0.541 (53)	2.806	1.802 (64)	1.004 (36)	3.391	2.219 (65)	1.172 (35)

The labelling period was 5 min and the concentration of CB was 10 μg/ml, with a concentration of DMSO of 0.25%. The same concentration of DMSO was present in the labelling medium used to measure the total uptake in the ' ence of CB. The CB-sensitive uptake was calculated by substracting the CB-resistant uptake from the total uptake. The concentration of [^{3}H]-2dg was 13 mM, corresponding to 0.25 μCi per ml, and that of [^{14}C]-Fdg was 380 nM, corresponding to 0.14 μCi per ml. Final hexose concentrations were adjusted by addition of D-glucose.

facilitated diffusion was responsible for the higher hexose uptake by transformed cells. At a glucose concentration of 5.5 mM, when differential uptake between normal and transformed cells was no longer noticeable, the respective contributions of simple diffusion (CB-resistant) and facilitated diffusion (CB-sensitive) uptakes were of the same order of magnitude in normal and transformed cells. This eliminated the possibility that a high level of simple diffusion might have hidden a difference in the efficiency of the carrier-mediated (facilitated mode) transports.

Little is known about the ultimate fate of non-metabolisable hexose analogues once trapped inside the cells by phosphorylation. We tested whether a difference in the retention of [^{14}C]-Fdg inside the cells might help to differentiate transformed from normal cells under physiological conditions. Cells were labelled for 15 min with [^{14}C]-Fdg in complete tissue culture medium whose glucose concentration was 5.5 mM; after washing out the labelling medium the cultures were replenished with the same medium without [^{14}C]-Fdg, and further incubated for 2 hr at 37°C before determining the amount of [^{14}C]-Fdg still present in the cells compared to that present at the end of the initial labelling period. For both chicken and mouse cells, no differential loss of [^{14}C]-Fdg from normal or transformed fibroblasts was observed (Table 4).

Finally, we tested if a longer labelling period could help to show up a differential uptake and retention of [^{14}C]-Fdg between normal and transformed cells (Table 5). Cultures were incubated either for 2 hr in complete medium containing 5.5 mM of glucose and 0.14 μCi per ml of [^{14}C]-Fdg or for 18 hr in medium with only 0.014 μCi per ml of the radioactive sugar at initial glucose concentrations of 5.5 and 22 mM, respectively. This latter radioactive concentration has no toxic effect during the 18-hr period, whereas the higher activity used in short-term experiments would have exerted a radiotoxic effect on the cells. In these conditions the labelling of normal and transformed cells was virtually the same, except in the case of chicken cells maintained for 18 hr in the medium with an initial glucose concentration of 5.5 mM, where the uptake of transformed CEF was about twice that of the control cells. But the glucose concentration had dropped from the initial 5.5 mM to 90 μM and 70 μM, for normal and transformed CEF respectively, during this period. This lowering of the glucose concentration to well below physiological conditions [17] would allow expression of the differential uptake. Indeed, as shown in Table 1, in short-term experiments conducted with [^{3}H]-2dg, lowering the glucose concentration from 5.5 to 2 mM is already sufficient to reveal a higher uptake by transformed CEF. Therefore, such a result is more likely an artifact due to tissue culture conditions rather than an indication of an actual differential uptake under physiological conditions.

DISCUSSION

The above results show that 2-fluorodeoxyglucose is an equally efficient tracer of hexose uptake in tissue culture as is 2-deoxyglucose. For these two analogues and for the physiological sugar D-glucose, the difference in uptake between normal and transformed cells was only visible at low non-physiological hexose concentrations in the medium. Modification of labelling time or of post-labelling incubation did not affect the fact that no differential hexose uptake or retention was observed under an

Table 4. Retention of [^{14}C]-Fdg by normal or transformed chicken and mouse cells

	[^{14}C]-Fdg uptake and retention (pmol per 10^6 cells)			
	Chicken cells		Mouse cells	
	CEF	SR-A RSV CEF	C127	C127-Ki
End of the 15-min labelling period	4.180	4.646	7.957	4.867
After 2 hr incubation following the labelling period	3.200	3.556	6.849	3.229

Cell cultures were labelled during 15 min in complete medium containing 5.5 mM D-glucose and 0.14 μCi per ml of [^{14}C]-Fdg. At the end of the labelling period triplicate plates were processed for determining the hexose uptake, and parallel plates were further incubated for 2 hr in medium without radioactive sugar to determine the retention of the trapped [^{14}C]-Fdg.

Table 5. [^{14}C]-*Fdg uptake by normal and transformed cells over prolonged labelling periods under physiological conditions of glucose concentration*

Labelling period (hr)	Hexose concentration in the medium	[^{14}C]-Fdg uptake (pmol per 10^6 cells)					
		Chicken cells			Mouse cells		
		CEF	SR-A RSV CEF	Ratio SR-A RSV CEF: CEF	C127	C127-Ki	Ratio C127-Ki: C127
2	5.5 mM glucose + 0.14 μCi/ml [^{14}C]-Fdg	9.905	8.238	0.83	8.459	8.295	0.98
18	5.5 mM glucose + 0.14 μCi/ml [^{14}C]-Fdg	5.288	9.765	1.85	0.826	0.767	0.93
18	22 mM glucose + 0.014 μCi/ml [^{14}C]-Fdg	2.362	1.995	0.84	0.554	0.345	0.62

Triplicate cultures of chicken or mouse cells were labelled in complete medium containing the indicated concentrations of [^{14}C]-Fdg and D-glucose. During each of the labelling periods the external glucose concentration did not drop below 3 mM, except in the case of chicken cells maintained for 18 hr at an initial concentration of 5.5 mM (see text).

external glucose concentration corresponding to that of blood. Unfortunately, this *in vitro* study makes it unlikely that the accumulation of high levels of [^{18}F]-2-fluorodeoxyglucose might provide a means to detect *in vivo* tumoral localisations by external scanning. This pessimistic conclusion might be attenuated by the possibility that *in vitro* and *in vivo* cell behaviour could differ. Thus, the greater transport capacity of tumoral cells within poorly vascularised solid tumors might confer a selective advantage over surrounding normal cells.

Our data with chicken cells are quite comparable with those of Hatanaka and Hanafusa ([2]; see Table 2 in this ref.), which showed a progressive attenuation of the higher uptake by RSV-transformed CEF over normal cells as the external hexose concentration was increased, this higher uptake being no longer observed for substrate concentrations higher than 1 mM. They differ from those of Weber [6] and Kletzien and Perdue [7], who observed a greater uptake by transformed CEF than in normal cells over a range of substrate concentrations extending up to 10 mM or above. Our results with mouse cells are in overall agreement with those of Romano and Colby [10]. In our experiments the uptake of [^{3}H]-2dg or of [^{14}C]-Fdg in normal and transformed CEF on the one hand, and in normal and transformed mouse cells on the other hand, was inhibited at the physiological glucose concentration and in essentially similar proportions by cytochalasin B. These results indicated that hexose transport by saturable facilitated diffusion and by non-saturable simple diffusion were equally efficient in normal and transformed cells of both the chicken and mouse cell systems. It is likely that the progressive attenuation of the differential hexose uptake as a function of the increasingly higher initial concentration of glucose in the labelling medium reflected the saturation of the facilitated diffusion transport system. However, the possibility also exists that the level of hexokinases and the extent of hexose phosphorylation, which are considered by some authors [9, 10] but not by others [7] as the critical limiting step regulating the retention of hexoses entering the cells, could also interfere with the extent of the differential labelling according to the glucose concentration in the surrounding medium.

To conclude, there is a general agreement about the fact, though not about the actual transport mechanisms, that transformed cells possess a higher hexose uptake rate than the normal cells at low external hexose concentrations, but the persistence of such an enhanced uptake at substrate concentrations existing *in vivo* is subject to some variation. In this field of *in vitro* cell physiopathology, further clarification of the mechanisms behind these variations would help to orientate less ambiguously the *in vivo* applications of positron-emitting tracers and external scanning devices which are now available.

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