UPTAKE OF GLYCEROL BY TUMOR CELLS AND ITS CONTROL BY GLUCOSE

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SUMMARY: Glycerol can be utilized by cultured cells of Novikoff rat hepatoma, Hela and HEP-2. Glucose inhibits the rate of glycerol uptake by the first two cell lines but stimulates the process in the third. The transport process of glycerol, particularly by its insensitivity to phloridzin, is distinguishable from that of glucose. The inhibitory effect of glucose on glycerol uptake which is competitive in nature is at the membrane transport and not phosphorylation step, since in vitro glycerol kinase is sensitive to neither glucose nor hexose phosphates.

In prokaryotic cells, such as Escherichia coli, glycerol seems to enter the cell via a permease (facilitator) not coupled to metabolic energy (1-3). The substrate is trapped intracellularly by an ATP-dependent phosphorylation (4-8). Cultured MB III mouse lymphoblast cells have been reported to grow on glycerol instead of glucose as the principal carbon source, although the possibility of the existence of a glycerol permease was not raised (9). On the other hand, a stereospecific and copper ion inhibitable process for glycerol permeation has been found in erythrocytes of various animals (10-12), although such cells are not known to metabolize glycerol.

In the present study, Novikoff rat hepatoma, subline N1S1-67 (from P. E. W. Plagemann), a Hela subline (from D. Baltimore) and HEP-2 (from Flow Laboratories, Rockville, Md.) were observed to take up glycerol at significant rates. Our objective is to explore further whether a specific membrane system mediates glycerol uptake, and whether the rate of this process can be regulated by glucose.

MATERIALS AND METHODS: (^{14}C-U)-glycerol and glucose were purchased from New England Nuclear, Boston, Ma; Swim's 67-G medium from Grand Island Biological Co., Grand Island, N.Y.; and calf sera and all other special components used for culturing cells from the Flow Laboratories, Rockville, Md.

NlSl-67 cells were propagated in liquid culture in Swim's 67-G medium supplemented with 10% fetal calf serum; Hela cells in Joklik modified MEM supplemented with 7% calf serum; and HEP-2 cells in MEM supplemented with 10% fetal calf serum. For resuspension and incubation of cells in all uptake experiments, a solution designated as MEMG$^-$ was used and was prepared according to the formula of Joklik modified MEM with the following exceptions: (a) glucose and bicarbonate were omitted, (b) MEM nonessential amino acids were added, each at a final concentration of 2 mM and (c) dibasic instead of monobasic sodium phosphate was used.

For assays of substrate uptake, about 1-2 x 10^7 cells from a growing culture were harvested by centrifugation at 1000 x g for 5 min. The pellet was dispersed in 50 ml of MEMG$^-$. After recentrifugation, the cells were finally suspended in a total volume of 5 ml MEMG$^-$ and equilibrated in a large test tube immersed in a reciprocal shaking water bath at 37 C for 5 min. At various time intervals following the addition of the labeled substrate at 0.1 mM, 0.5 ml samples were delivered onto a Millipore filter disc (0.65 microns pore size) on a sintered glass platform (without chimney) connected to an aspirator. The collected cells were rinsed in situ with 5 ml phosphate buffered saline at room temperature. The disc was measured for radioactivity. A background value, obtained by extrapolation to zero time, was subtracted from each experimental value.

For the assay of glycerol kinase, about 10^8 cells suspended in 1.5 ml of 20 mM sodium acetate and 2 mM dithiothreitol at pH 5.8 were subjected to sonic disruption for 1.5 min in a model 60 W MSE ultrasonic disintegrater while chilled by ice. The cell extract was then passed through a G-25 sephadex column (1 x 24 cm) in the same buffer. Fifty µl of the eluent (about 30 µg protein) was assayed for activity in a total volume of 0.5 ml containing 20 mM glycylglycine-NaOH at pH 8.0, 5 mM $MgCl_2$, 10 mM ATP (neutralized) and 0.1 mM labeled glycerol. At various time intervals, 50 µl of the reaction mixture was placed onto a dry DEAE-cellulose paper disc (DE-81, W. and R. Balston, England); the filter disc was then dropped in 80% ethanol to stop the reaction, washed by filtration with 5 ml distilled water, and measured for radioactivity. The radioactivity retained on the disc, after appropriate corrections for blank values, represented the amount of the phosphorylated form of glycerol. Bovine albumin was used as the standard for measurements of protein concentration (13).

RESULTS AND DISCUSSION: All three lines of cells, NlSl-67, Hela and HEP-2 took up glycerol at similar and close to linear rates for at least 10 min (Fig.1), but the effect of glucose on glycerol uptake varied with the cell lines. Whereas the rate of glycerol uptake by NlSl-67 and Hela cells was inhibited by the hexose, the same activity in HEP-2 cells was slightly stimulated. None of the

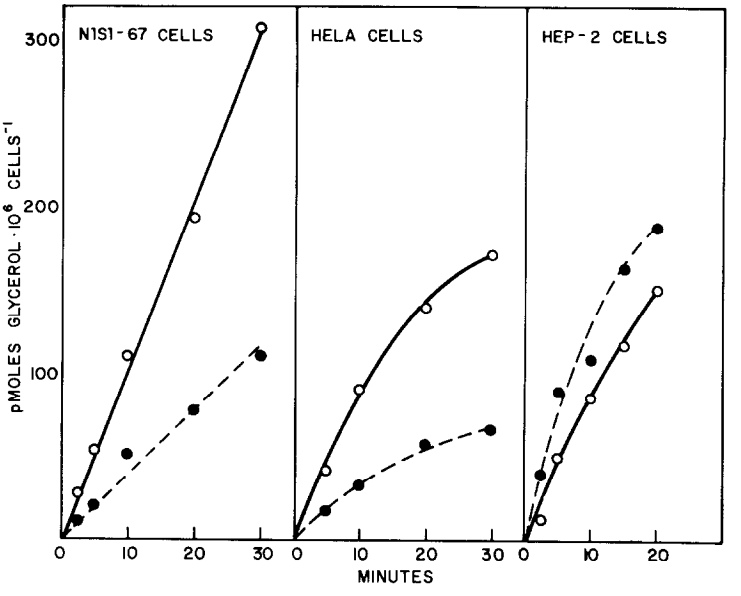

Figure 1. The rate of ^{14}C-glycerol uptake at 0.1 mM by three lines of mammalian cells in the presence ●- - -● or absence o——o of glucose at 5 mM.

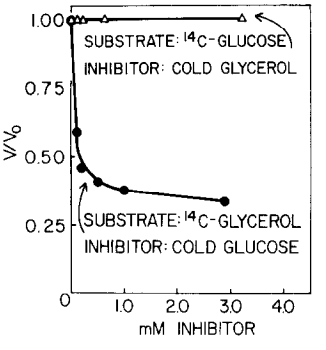

Figure 2. Inhibition by glucose of the glycerol uptake process in cells of N1S1-67 but not vice versa. In each case, the substrate for uptake was maintained at a constant concentration of 0.1 mM. The inhibitor concentration was varied as indicated.

cell lines grew on glycerol without glucose; hence the possible effects of the hexose on the actual level of the glycerol uptake systems could not be tested. Further studies of glycerol uptake were focused on N1S1-67 cells because of their rapid growth rate (generation time, 12 hr), their satisfactory rate of glycerol

Table 1. Kinetic properties of glycerol uptake and phosphorylation.

	K_m (μM)	V_{max} pmoles/mg/min	Inhibition by 5 mM glucose
Glycerol Uptake	100	60	70 %
Glycerol Kinase	4	140	0

uptake, and the ability of glucose to inhibit the process.

Although the inhibitory effect of glucose on glycerol uptake appears to be competitive, with a K_i of approximately 0.9 mM (data not shown), two lines of evidence indicate that glycerol enters the cell via a route separate from that for glucose. First, although the uptake of glycerol at 0.1 mM could be inhibited 50% by 0.1 mM glucose, the uptake of glucose at 0.1 mM by these cells was not affected at all by glycerol even at 3 mM (Fig.2). This nonreciprocal inhibition could not be explained by the operation of a common membrane carrier strongly preferring glucose because when the substrates were presented singly, glycerol uptake exhibited an apparent K_m of 0.1 mM (Table 1) and glucose uptake exhibited a K_m of 1-2 mM (14). Secondly, as shown in Fig. 3, although the uptake of glucose by the NlSl-67 cells was drastically inhibited by phloridzin, as one would expect from studies on other mammalian cells (15-19), the uptake of glycerol was not.

Since 1) glycerol kinase has been found to have a lower K_m for glycerol than that of the glycerol transport system (Table 1); 2) glycerol-3-phosphate is probably an obligatory intermediate in glycerol metabolism; and 3) during the uptake assay, cellular re-

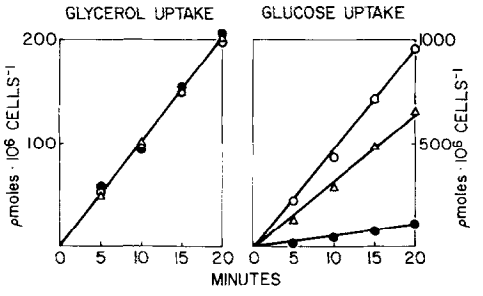

Figure 3. Effect of phloridzin on the rate of uptake of glycerol or glucose by N1S1-67 cells. o——o, no inhibitor added; Δ——Δ, 0.1 mM phloridzin; ●- - -●, 1.4 mM phloridzin.

tention of this anionic intermediate is more likely than that of its uncharged precursor, the possibility that glucose and its close metabolic products (i.e., glucose-6-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate) might inhibit glycerol utilization at the phosphorylation step was examined. When the activity of glycerol kinase was measured in vitro, none of these compounds showed any effect at a concentration 20 times higher than that of the substrate which was used at 0.1 mM (See Table 1. The rest of the data not shown).

In brief, it appears that glycerol enters the N1S1-67 cells through a mediated process distinct from that for glucose and that the hexose, the preferred nutrient, can exert negative kinetic control over the utilization of glycerol at the membrane level.

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