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ACCUMULATION AND METABOLISM OF 2-DEOXY-D-GLUCOSE-1-^{14}C IN THE HUMAN PLATELET

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Abstract—Human platelets incubated at 37° concentrate 2-deoxy-D-glucose-^{14}C (dGlc) against a gradient by a process which is saturable and restrained by metabolic inhibitors, other hexoses, and phlorizin. Insulin had no effect. Even though a gradient for dGlc was established, approximately 90 per cent of the total sugar within the platelet was present as dGlc-6-P. These data are consistent with an active transport system for this sugar within the cellular membrane and the absence of a dGlc-6-phosphatase suggests that such transport does not involve phosphorylation. The presence of free dGlc in the platelet shows the capacity for uptake exceeds, by a small margin, that of phosphorylation. Platelet homogenate, fortified with ATP (10^{-3}M) also phosphorylated dGlc. Such phosphorylation was inhibited by D-glucose, D-mannose, and D-fructose but not by dGlc-6-P. Since uptake by the intact platelet virtually ceased when the intraplatelet concentration of dGlc-6-P approached 2×10^{-3}M, the rate of uptake of dGlc may be controlled by the intraplatelet level of dGlc-6-P.

CLOT retraction, subsequent to the treatment of platelets with thrombin, requires energy.[1] The energy apparently derives from glucose metabolism since, in its absence, retraction does not occur. Furthermore, such retraction is impaired in the presence of glycolytic inhibitors.[2] Although glucose metabolism has been extensively studied in this cell,[1] little is known of the mechanism or control of its penetration.

The rapid metabolism of glucose by the platelet precludes the use of this sugar in characterizing its own penetration. Therefore, 2-deoxy-D-glucose was chosen for such characterization since its metabolism is restricted to phosphorylation at the 6-position.

EXPERIMENTAL SECTION

Platelet-rich plasma was obtained from healthy human donors as previously described.[3] Approximately 35 mg wet weight of cells were sedimented, washed twice with Tris-buffered saline (pH 7·5) containing 1·3 mM EDTA, and resuspended in 0·9 ml of Krebs–Ringer bicarbonate buffer (pH 7·4) from which the calcium and magnesium ions had been omitted. Samples were incubated for varying periods of time at 37° in a Dubnoff metabolic shaker with 0·1 ml of 2-deoxy-D-glucose-1-^{14}C (dGlc) (0·1 μc). After incubation, the platelets were sedimented, supernatant solutions decanted, and the tubes swabbed with a cotton-tipped applicator. The samples were weighed and lysed in 1 ml of distilled water. dGlc-1-^{14}C and 2-deoxy-D-glucose-1-^{14}C-6-phosphate (dGlc-6-P) in the supernatants and lysates were separated by the $Ba(OH)_2$-$ZnSO_4$ precipitation method of Somogyi[4] or by chromatography (TLC) on

thin layers of cellulose according to the method of Weidemann and Fischer.[5] In the latter studies, "carrier" dGlc and dGlc-6-P (0·2 mg/ml) were added to the platelet lysate and medium prior to chromatographic separation. The compounds were visualized by spraying with perchloric acid in acetone.[5] The cellulose, along with the compounds, was scraped from the plates, suspended in gelled scintillation medium[6] and the radioactivity determined. The distribution ratio ([I]/[O]) of dGlc was expressed as the ratio of disintegration per minute per milliliter of platelet water to disintegration per minute per milliliter of incubation medium. Total platelet water was 76 per cent and extracellular water 27 per cent of the wet weight of the platelet pellet.[7] The standard error of both determinations was ± 5 per cent and, to this extent, such errors would be reflected in the distribution ratios. The concentration of dGlc in the intraplatelet water was corrected for that trapped within the extracellular space of the platelet pellet according to the formulation of Helmreich and Kipnis.[8] In all measurements of radioactivity, samples were counted for sufficient time to accumulate 10,000 counts. Radioactivity of all samples exceeded ten times background. Counting efficiency determined by the channels ratio method was 72–76 per cent. dGlc-1-^{14}C (sp. act., 7·85 mc/mM), obtained from New England Nuclear Corp., was found to be >98 per cent pure by thin-layer chromatography.[5]

In some experiments the influence of various agents on the uptake of dGlc by the platelet was studied. These included phlorizin, iodoacetic acid, dinitrophenol (DNP), sodium cyanide (NaCN), sodium fluoride (NaF), *p*-chloro-mercuribenzoic acid (PCMB), and bovine insulin. In other studies the effects of a variety of unlabeled sugars were studied on the uptake of ^{14}C-dGlc. These sugars included dGlc, D-glucose, L-glucose, D-mannose, D-fructose, D-ribose, 2-deoxy-D-ribose, L-xylose, D-arabinose, D-fucose, D-galactose, 2-deoxy-D-galactose, 3-*O*-methyl-D-glucopyranose, methyl-alpha-D-glucopyranoside, and i-inositol.

In some experiments, platelets (40 mg) were homogenized in isotonic saline (1 ml) with a Ten Broeck tissue grinder and the homogenates incubated for 1 hr at 37° with ^{14}C-dGlc (0·1 μc) and ATP (10^{-3}M). dGlc-6-P-1-^{14}C in the incubation medium was precipitated by addition of the barium hydroxide–zinc sulfate reagent of Somogyi.[4] The extent of phosphorylation of ^{14}C-dGlc was determined by measuring the radioactivity of aliquots of the incubation medium prior to and after addition of the Somogyi reagent. Radioactivity in these samples was determined by liquid scintillation spectrometry as previously described.

RESULTS

Uptake of dGlc by the intact platelet. Human platelets concentrated dGlc against a gradient; the steady state distribution ratio ([I]/[O]) approached a maximum of 9·3, within 15 min of incubation, at an initial dGlc concentration of $2{\cdot}0 \times 10^{-5}$M (Fig. 1). Increasing the concentration of dGlc progressively reduced the gradient such that the value was only 1·5 at an initial concentration of $5{\cdot}3 \times 10^{-4}$M (Fig. 1). Uptake of this sugar was markedly reduced in the presence of phlorizin (10^{-3}M) (Fig. 2) but was not influenced by bovine insulin (0·4 U/ml).

The effect of various metabolic inhibitors on the uptake of dGlc by the platelet is shown in Table 1. The steady state distribution ratio of this sugar, at an initial concentration of $1{\cdot}4 \times 10^{-4}$M, was reduced from 14·2 to 2·1 in the presence of iodoacetic

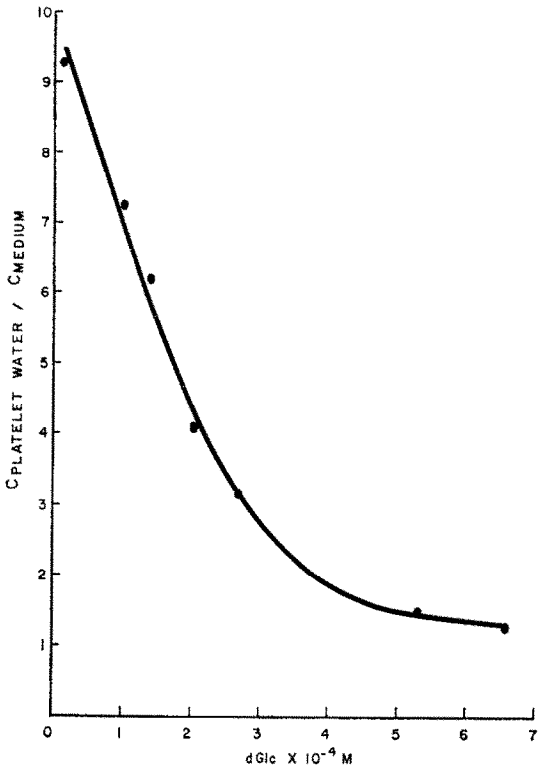

FIG. 1. Steady-state distribution of free dGlc in the human platelet when incubated with various concentrations of this sugar for 15 min at 37°. Results of a representative experiment are shown.

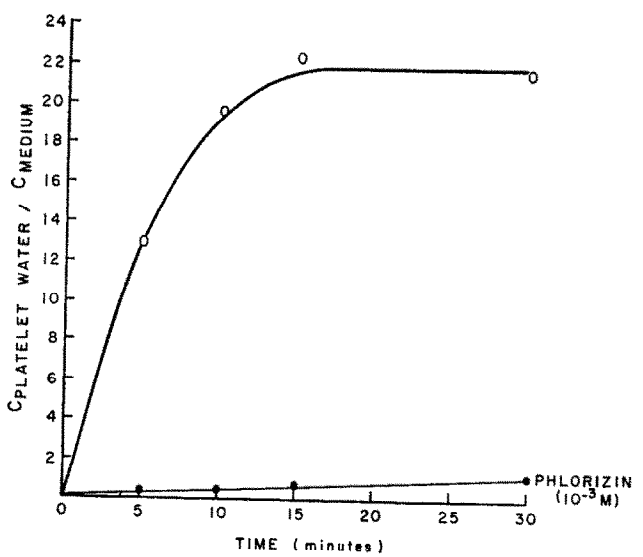

FIG. 2. The accumulation of free dGlc ($1{\cdot}4 \times 10^{-4}$M) by the human platelet incubated at 37° in the presence and absence of phlorizin. Results of a representative experiment are shown.

acid (10^{-3}M). Accumulation was also diminished by PCMB, DNP, and NaCN but not by NaF.

Other sugars were also assessed as inhibitors of uptake of dGlc ($1{\cdot}4 \times 10^{-4}$M) (Fig. 3). dGlc itself was the most potent with 50 per cent inhibition at 2×10^{-4}M. Approximately 50 per cent inhibition also occurred with D-mannose at 7×10^{-4}M, D-glucose at $1{\cdot}2 \times 10^{-3}$M, and D-fructose at 6×10^{-3}M. Under the same conditions, 3-*O*-methyl-D-glucopyranose ($6{\cdot}5 \times 10^{-3}$M) produced 20 per cent inhibition and

TABLE 1. INFLUENCE OF VARIOUS INHIBITORS (10^{-3}M) ON THE ACCUMULATION OF FREE dGlc ($1{\cdot}4 \times 10^{-4}$M) BY THE HUMAN PLATELET WHEN INCUBATED FOR 15 min AT 37°

Inhibitor	Gradient*	Per cent inhibition of uptake
Control	$14{\cdot}2 \pm 1{\cdot}8$	
DNP	$11{\cdot}2 \pm 1{\cdot}1$	21·1
Iodoacetic acid	$2{\cdot}1 \pm 0{\cdot}4$	85·2
NaF	$13{\cdot}8 \pm 1{\cdot}4$	2·8
NaCN	$12{\cdot}0 \pm 1{\cdot}9$	15·5
PCMB	$9{\cdot}5 \pm 0{\cdot}7$	33·1

* Gradient = Concentration in intraplatelet water per concentration in incubation medium. Mean ± S. E. of four experiments.

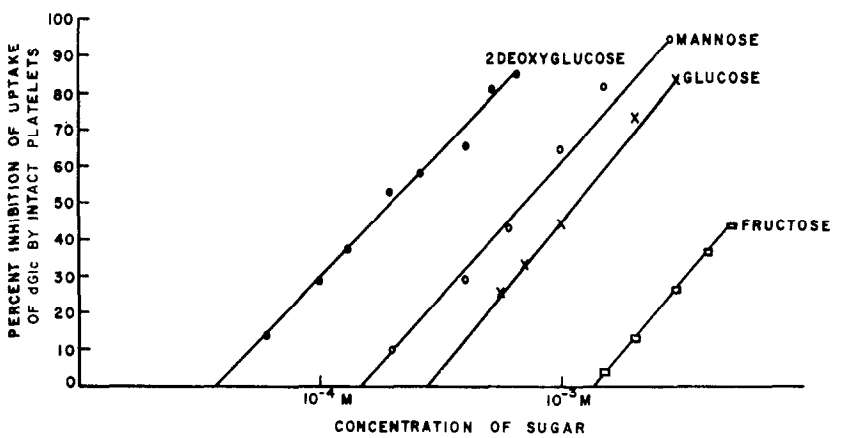

FIG. 3. Inhibition of accumulation of ^{14}C-dGlc (free) by nonradioactive dGlc and other hexoses. The concentration of ^{14}C-dGlc was $1{\cdot}4 \times 10^{-4}$M in all experiments. Platelets were incubated for 15 min at 37°. Results of representative experiments are shown.

methyl-alpha-D-glucopyranoside ($6{\cdot}5 \times 10^{-3}$M), 10 per cent. At a concentration of $6{\cdot}5 \times 10^{-3}$M, the following carbohydrates did not inhibit uptake of dGlc ($1{\cdot}4 \times 10^{-4}$M): i-inositol, D-ribose, 2-deoxy-D-ribose, L-xylose, D-arabinose, L-arabinose, D-fucose, D-galactose, 2-deoxy-D-galactose, and L-glucose.

Since the total uptake of dGlc-^{14}C was far in excess of that attributable to unchanged dGlc, phosphorylation was considered and confirmed by TLC and differential precipitation. Indeed, all the radioactivity was confined to dGlc-6-P and

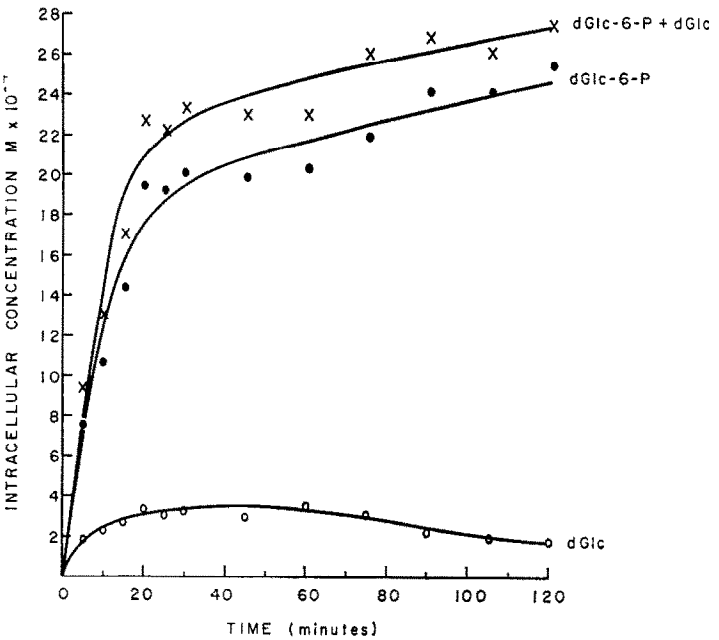

FIG. 4. Total rate of penetration of dGlc and rates of accumulation of free dGlc and dGlc-6-P by the human platelet. Platelets were incubated at 37° with dGlc at an initial concentration of $1{\cdot}4 \times 10^{-4}$M. The concentration of dGlc in the medium stabilized at $7{\cdot}3 \times 10^{-5}$M within 15 min. Results of a representative experiment are shown.

unchanged dGlc. The rates of phosphorylation and penetration of dGlc into the platelet were virtually linear with time up to 20 min of incubation (Fig. 4). During this phase, the rate of entry approximated $8{\cdot}4 \times 10^{-5}$ μM dGlc/mg platelet/min and of phosphorylation, $7{\cdot}2 \times 10^{-5}$ μM dGlc/mg platelet/min. Shortly after the twentieth minute of incubation, when the intracellular concentration of dGlc-6-P was $1{\cdot}9 \pm 0{\cdot}2 \times 10^{-3}$M (mean $\pm$S. E. of 6 experiments), a sharp decline in these rates and a progressive decrease in the intraplatelet concentration of dGlc ensued (Fig. 4). Apparently, insulin does not influence phosphorylation or uptake since the intraplatelet concentration of dGlc and dGlc-6-P did not change in the presence of this hormone.

The possibility of a dGlc-6-phosphatase in the platelet was also assessed. After the cells were incubated for 1 hr with ^{14}C-dGlc ($1{\cdot}4 \times 10^{-3}$M), iodoacetic acid (10^{-3}M) was added prior to a second hour of incubation. Although accumulation and phosphorylation ceased, the amount of ^{14}C-dGlc-6-P in the platelet and ^{14}C-dGlc in the platelet and incubation medium remained the same. Moreover, when the medium was replaced with that containing nonradioactive dGlc ($1{\cdot}4 \times 10^{-5}$M) for a second hour of incubation, the amount of intraplatelet ^{14}C-dGlc-6-P was not changed. Although under these conditions some loss of intraplatelet ^{14}C-dGlc to the medium ensued, the amount of ^{14}C-dGlc in the total incubate remained the same. These data indicate the absence of d-Glc-6-phosphatase and preclude the possibility that dGlc-6-P is a precursor of dGlc within the platelet.

Phosphorylation of dGlc by platelet homogenates. Platelet homogenates, fortified with ATP (10^{-3}M), also phosphorylated dGlc at a rate dependent upon the concentration of substrate and time. At a dGlc concentration of $6{\cdot}5 \times 10^{-4}$M, phosphorylation was inhibited approximately 50 per cent by D-glucose (10^{-4}M), dGlc ($2{\cdot}5 \times 10^{-3}$M), D-mannose ($2{\cdot}5 \times 10^{-3}$M), and D-fructose ($1{\cdot}5 \times 10^{-2}$M) (Fig. 5). Of these hexoses, D-glucose had the greatest affinity for the enzyme and D-fructose the least. In this system, phosphorylation was not inhibited by dGlc-6-P (2×10^{-3}M) where the concentration of dGlc was rate limiting.

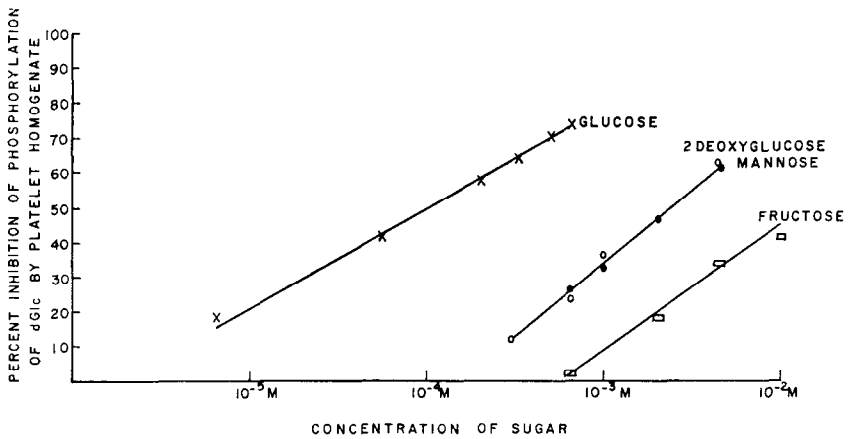

FIG. 5. Inhibition of ^{14}C-dGlc phosphorylation by nonradioactive dGlc and other hexoses in platelet homogenate fortified with ATP (10^{-3}M). The concentration of ^{14}C-dGlc was $6{\cdot}5 \times 10^{-4}$M in all experiments. Homogenates were incubated for 15 min at 37°. Results of representative experiments are shown.

DISCUSSION

In this study, evidence is presented that the human platelet concentrates ^{14}C-dGlc against a gradient by a process which is saturable and restrained by metabolic inhibitors, other hexoses, and phlorizin. Even though a gradient for dGlc was established, approximately 90 per cent of the total sugar within the platelet was present as dGlc-6-P. These data are consistent with an active transport system for this sugar within the cellular membrane and the absence of a dGlc-6-phosphatase suggests that such transport does not involve phosphorylation. The presence of free dGlc in the platelet shows the capacity for uptake exceeds that of phosphorylation. However, such differences in capacity are small; during the first 20 min of incubation, when the rates of phosphorylation and accumulation were linear with time, approximately 90 per cent of dGlc which entered was phosphorylated. Such phosphorylation is consonant with Murer's study in which dGlc-6-^{32}P was isolated from human platelets incubated with ^{32}Pi and dGlc.[9] At several concentrations of dGlc in the incubation medium, Kipnis and Cori[10] could not demonstrate the presence of free dGlc inside the muscle cell unless glucose or mannose was added to the medium. Apparently these sugars compete with dGlc for hexokinase.[10] Their data suggest that, unlike the platelet, the maximum capacity of muscle to phosphorylate this sugar is greater than the rate of penetration.

A number of cell types such as yeast,[11] kidney cortex slices,[12] ascites cells,[13] and lymph node cells of the guinea pig[14] have been shown to accumulate dGlc. However, yeast, unlike the human platelet, contains dGlc-6-phosphatase and, in this cell, dGlc-6-P behaved as the precursor of intracellular free dGlc.[4]

Uptake of dGlc by the platelet resembles that of the isolated lymph node cell.[14] Apparently, D-mannose, D-glucose and D-fructose share a transport system with dGlc in both cells[14] since these sugars inhibit the uptake of dGlc. Furthermore, in these cells, such uptake markedly decreased as the intracellular level of dGlc-6-P increased. The latter data may be explained in several ways.

A likely possibility lies in feedback inhibition of dGlc uptake at the membrane by dGlc-6-P. Entry of ^{14}C-dGlc virtually ceased when the intraplatelet level of dGlc-6-P approached 2×10^{-3}M, whereas dGlc-6-P, at the same concentration, did not inhibit phosphorylation of dGlc in the platelet homogenate. The much slower but persistent phosphorylation, after 20 min of incubation when the concentration of intraplatelet dGlc-6-P was approximately 2×10^{-3}M, progressed at the expense of residual intraplatelet dGlc rather than that of continued entry. Hence, these data suggest that glucose metabolism is controlled in part by the intraplatelet concentration of glucose 6-phosphate which, when high, suppresses further transport of glucose into the cell. This hypothesis is particularly attractive since the uptake process is not rate limiting for phosphorylation.

Inasmuch as dGlc uptake and phosphorylation are ATP dependent, a second possibility lies in depletion of this energy source by dGlc. However, such depletion seems unlikely in our study since Schneider and Niemeyer[15] found very little reduction in ATP levels in human platelets incubated for 60 min with dGlc (10^{-3}M).

The inhibition of platelet hexokinase by D-glucose, D-mannose, and D-fructose, and the small or complete lack of inhibition by 3-*O*-methyl-D-glucopyranose, methyl-alpha-D-glucopyranoside, D-arabinose, D-galactose, D-ribose, and i-inositol, are consistent with the relative maximum rates of phosphorylation of these sugars reported for brain and ascites tumor hexokinase.[16,17] The stereo-specificity of platelet hexokinase is further documented by the lack of inhibition of dGlc phosphorylation by L-glucose.

Karpatkin reported lactate production in the human platelet to be approximately doubled in the presence of insulin and glucose as compared to glucose alone.[18] Since intracellular glucose was not detectable, its rate of disappearance from the medium presumably reflected the rate of utilization. He, therefore, suggested that insulin enhanced glucose transport. In our study, however, the uptake of dGlc by the human platelet was not influenced by insulin. In this regard the platelet resembles brain[19] but not muscle.[10]

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