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Glucose Uptake into Plasma Membrane Vesicles from the Maternal Surface of Human Placenta

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Summary. Glucose uptake into plasma membrane vesicles from the maternal surface of the human placenta was measured with the Millipore filtration technique. Uptake of D-glucose was dependent on the osmolarity of the incubation medium surrounding the vesicles. Uptake of D-glucose exceeded that of L-glucose. The uptake of D-glucose was not enhanced by placing 100 mM NaCl or NaSCN in the medium outside the vesicles (none inside) at the onset of uptake determinations. D-glucose transport was inhibited by cytochalasin B; phloretin, phlorizin, and 1-fluoro-2,4-dinitrobenzene. D-glucose uptake was inhibited by 2-deoxy-D-glucose, 3-O-methyl-D-glucose and to a lesser extent by D-galactose. It was not inhibited by α-methyl-D-glucoside. Cytochalasin B binding to the vesicles was 30% inhibited in the presence of 80 mM D-glucose. The results indicate that the system for facilitated transport of D-glucose at the maternal face of the placenta is distinctly different from that on the brush-border membrane of intestine or renal tubule and more closely resembles that of human erythrocyte.

Over twenty-five years ago, Widdas (1952) concluded from an analysis of the data of Huggett, Warren and Warren (1951) that glucose transport across the placenta of sheep was mediated by a carrier. Since that time a number of studies in the human placenta have supported the hypothesis for facilitated diffusion of glucose. *In vivo* studies have shown that fetal plasma glucose levels do not increase linearly with an increase in concentration gradient across the placenta (Cordero, Yen, Grunt & Anderson, 1970; Oakley, Beard & Turner, 1972). This observation is consistent with partial saturation of a carrier mechanism. Artificially perfused human placental studies have shown *in vitro* that glucose transfer is stereospecific (Carstensen, Leichtweiss, Molsen & Schröder, 1977), that D-glucose transfer can be competitively inhibited by D-mannose (Carstensen et al., 1977) and 3-O-methyl-α-D-glucopyranoside (Rice, Rourke & Nesbitt, 1976), and that counter transport of glucose may be demonstrated (Rice, Nesbitt & Rourke, 1976). In addition, glucose transport showed saturation kinetics (Rice et al., 1976) although this finding could not be confirmed in a subsequent study (Carstensen et al., 1977).

Preparation of plasma membrane vesicles from human placental syncytiotrophoblast has been demonstrated (Smith, Brush & Luckett, 1975; Smith, Nelson, King, Donahue, Ruzycki & Kelly, 1977). The use of the vesicle system has the advantage of eliminating metabolism and offers the ability to control composition of the solutions on both sides of the membrane, compared to intact cells or the whole organ. We have studied glucose uptake into plasma membrane vesicles prepared from the maternal face of human placental trophoblast cells in order to characterize the glucose transport system. We have evaluated the role of sodium in placental glucose transport. In addition we have examined the effects of the inhibitors: phlorizin, phloretin, fluorodinitrobenzene (FDNB) and cytochalasin B (CB) on glucose transport. Finally, we have studied the effect of D-glucose analogs on the uptake of D-glucose into the vesicles.

Materials and Methods

Preparation of Vesicles

Plasma membrane vesicles from the human placenta were prepared as described (Smith et al., 1975; Smith et al., 1977) with modifications adapted from procedures applied to the kidney (Turner & Silverman, 1977). Placentas from normal pregnancies of 37–

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41 weeks gestation were obtained within 1 hr of delivery. *Step 1:* 100 g of tissue were cut from the maternal surface and minced well with fine scissors. All subsequent steps were carried out at 4 °C. *Step 2:* the tissue was washed three times in a 200-ml volume of 100 mM $CaCl_2$. *Step 3:* the tissue was washed three times in a 200-ml volume of 10 mM triethanolamine hydrochloride (TEA-HCl), 250 mM sucrose. *Step 4:* the tissue was placed in 150-ml volume of 10 mM TEA-HCl, 250 mM sucrose and agitated with a magnetic stirring bar for 30 min. *Step 5:* the tissue was filtered through four layers of gauze. *Step 6:* filtrate 5 was centrifuged at $800 \times g$ for 10 min. *Step 7:* supernatant 6 was centrifuged at $10{,}400 \times g$ for 20 min. *Step 8:* supernatant 7 was centrifuged at $105{,}000 \times g$ for 30 min. *Step 9:* pellet 8 was taken up in 100 mM D-mannitol, 1 mM N-2-hydroxyethylpiperanzine-N'-2-ethane-sulfonic acid-Tris buffer, pH 74. (Tris-HEPES), 1 mM $MgCl_2$ (Buffer A), through a 25-gauge needle and passed twice through a 30-gauge needle. *Step 10:* suspension 9 was placed in a beaker and diluted to 80 ml with Buffer A. $MgCl_2$ was added to give a final concentration of 10 mM. The suspension stood on ice for 10 min with occasional stirring. *Step 11:* suspension 10 was centrifuged at $2000 \times g$ for 10 min. *Step 12:* supernatant 11 was centrifuged at $48{,}000 \times g$ for 20 min. *Step 13:* pellet 12 was resuspended in 1 ml Buffer A by passing twice through a 25-gauge needle. *Step 14:* suspension 13 was centrifuged at $30{,}000 \times g$ for 20 min. *Step 15:* pellet 14 was resuspended in 1–1.5 ml Buffer A by passing twice through a 30-gauge needle. *Step 16:* suspension 15 was incubated for 15 min at 37 °C and then stored on ice until use.

Glucose Uptake Studies

Uptake of labeled sugars into the plasma membrane vesicles was measured by the Millipore filtration technique (Carter, Avruch & Martin, 1972). Fifty µl of the vesicles were brought to 22 °C for 2 min. Uptake was initiated by the addition of 200 µl of incubation medium (IM). The incubation medium consisted of Buffer A with 1 mM each of D-glucose and L-glucose (containing 15–25 µCi/ml D-[^{14}C] glucose and 30–45 µCi/ml L-[^{3}H] glucose). Uptake was stopped by withdrawing 20 µl and placing it in 1 ml ice-cold stop solution (10 mM Tris-HEPES, 250 mM NaCl and 2mM phlorizin or 0.2 mM phloretin). The vesicles were rapidly separated by suction through 0.45-µm Millipore filters (HAWP 02500, Millipore Corporation, Bedford, MA). The filters were washed with 10 ml stop solution and placed in 15 ml commercially available liquid scintillation fluid (Biofluor, New England Nuclear, Boston, MA). The vials were stored overnight and counted for 10 min the following day. An external standard was used to provide corrections for efficiency and cross-over. Background filters were prepared by identical steps except that the vesicles were omitted. The DPM for these backgrounds, which average 7.4 ± 1.3 percent (mean ± SEM) of the activity of the uptake filters, were subtracted. Uptake was normalized with respect to mg of membrane protein and calculated from the specific activities of D-glucose and L-glucose in the incubation medium.

Additions to the incubation medium were made as shown in the Results. In the case of phlorizin, phloretin and glucose analogs there was no preincubation. For fluorodinitrobenzene and cytochalasin B the vesicles were preincubated with the inhibitor for 5 min at 22 °C prior to beginning the uptake study. Preliminary studies of these latter two inhibitors with preincubations of 1, 2, 5 and 30 min showed maximum effect was reached at 2 min.

Cytochalasin B Binding

Binding of cytochalasin B (CB) to the membrane vesicles was determined using methods similar to the glucose uptake studies except that the experiments were carried out at 4 °C. 12.5 µl of incubation medium containing 47.5 µCi/ml ^{3}H-CB was added to 135 µl of vesicles to bring the final concentration of CB to 0.33 µM. The final concentration of D-glucose was 80 mM and L-glucose at this concentration was present in the control experiments. After 5 min of preincubation the vesicles were separated and counted as described for the glucose uptake studies. Binding to filters prepared in the absence of vesicles averaged 12.6 ± 1.9% of the DPM seen in the presence of vesicles.

All values reported are the mean ± SEM of four experiments from vesicles prepared from an individual placenta. Each protocol was performed on vesicles from 2–4 placentas. Protein concentration was determined by the method of Lowry, Rosebrough, Farr and Randall (1951) and alkaline phosphatase as described by Bowers and McComb (1966).

The following isotopes were obtained from New England Nuclear Corporation, Boston, MA: glucose, D-[^{14}C(U)] 291.6–360 mCi/mmol; glucose, L-[1-^{3}H(N)] 17.46–18 Ci/mmol; and cytochalasin B, [4–^{3}H] 15 Ci/mmol.

Results

The final membrane preparation showed a homogeneous population of vesicles when examined by transmission electronmicroscopy. The majority of vesicles were 0.15–0.17 µm in diameter. Alkaline phosphatase, a marker for the maternal surface of the trophoblast (Hulsteart, Torringa, Koudstaal, Hardouk & Malenaar, 1973), was enriched 32.2 ±3.2-fold over the

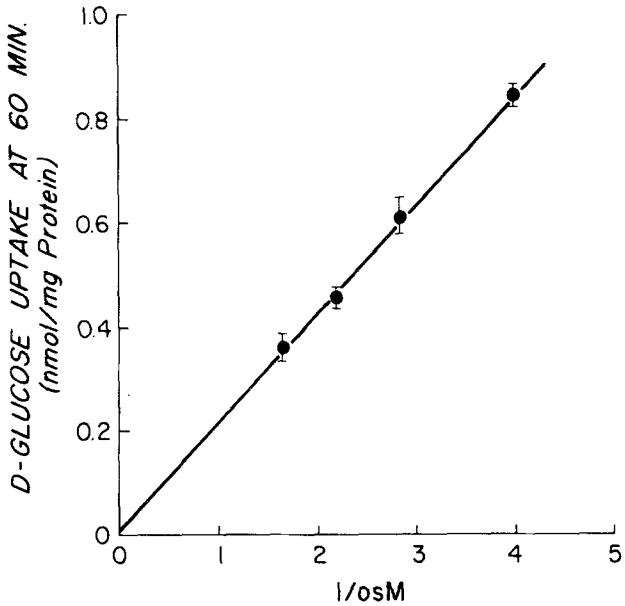

Fig. 1. Effect of incubation medium osmolarity on D-glucose uptake. 50 µl of placental plasma membrane vesicles, 5.0 mg protein/ml, in Buffer A (100 mM mannitol, 1 mM Tris-HEPES, 1 mM $MgCl_2$) were incubated with 200 µl incubation medium which consisted of buffer A plus 1 mM D-glucose (with 15 µCi/ml D-[^{14}C] glucose) and sucrose added to produce the osmolarities indicated on the abscissa. After 60 min a 50-µl aliquot was removed and separated by Millipore filtration. Values are the mean ± SEM of 4 experiments

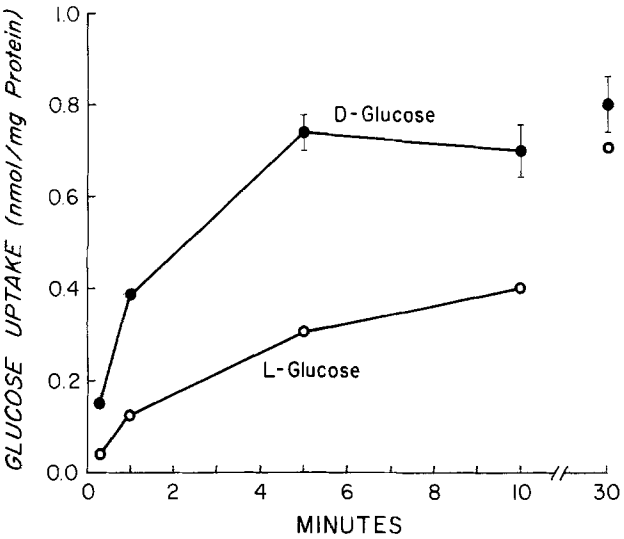

Fig. 2. Timed uptake of D-glucose (closed circles) and L-glucose (open circles). 50 µl of placental plasma membrane vesicles, 10.2 mg protein/ml, in Buffer A were incubated with 200 µl incubation medium which consisted of Buffer A plus 1 mM D-glucose (with 15 µCi/ml D-[^{14}C] glucose) and 1 mM L-glucose (with 25 µCi/ml L-[^{3}H] glucose). 20-µl aliquots were removed at 0.25, 1, 5, 10 and 30 min and the vesicles separated by Millipore filtration. Values are mean ± SEM of 4 experiments. Error bars are omitted if the symbol covers the extent of the SEM

homogenate of the initial placental tissue. The suspension of vesicles used for uptake studies contained 6.0 ± 0.6 mg protein per ml.

In order to test whether we were observing transport from the incubation medium into an intravesicular space as opposed to binding of the sugars to the vesicles (Carter et al., 1972) we measured the uptake of D-glucose at 60 min, with increasing medium osmolarity. The osmolarity was varied by addition of sucrose (Carter et al., 1972). The results are shown in Fig. 1. It can be seen that uptake decreases as a linear function of the reciprocal of medium osmolarity. In addition, extrapolation of this relationship shows that there would be no uptake at an infinite osmolarity indicating that there is no binding of D-glucose to the membrane vesicles.

Table 1. Effect of sodium on D-glucose uptake

Additions to incubation Medium	Uptake at 15 sec nm/mg protein		
	D-glucose	L-glucose	Net D-glucose
100 mM NaCl	0.305 ± 0.050	0.161 ± 0.028	0.144 ± 0.024
100 mM KCl	0.305 ± 0.039	0.122 ± 0.033	0.183 ± 0.036
100 mM NaSCN	0.228 ± 0.004	0.105 ± 0.006	0.123 ± 0.005
100 mM KSCN	0.255 ± 0.012	0.129 ± 0.012	0.126 ± 0.009

Uptake of D-glucose and L-glucose into vesicles at 15 sec measured as described in Fig. 2. Incubation medium contained, in addition, 100 mM of either NaCl, KCl, NaSCN or KSCN as indicated. Values are mean ± SEM of 4 experiments. Vesicles for the Cl^- and the SCN^- experiments were prepared from different placentas

Timed uptake studies of D- and L-glucose are shown in Fig. 2. Uptake of D-glucose exceeded that of L-glucose over the first 10 min with equilibrium nearly complete at 30 min. Timed uptake studies were also performed using either 1.0 or 0.1 mM D- and L-glucose in the incubation medium. Uptake was determined at 3, 7, 10 and 15 sec and during these early time points the net rate of D-glucose uptake was directly proportional to the concentration of glucose in the incubation medium. We concluded that there is not partial saturation of the carrier system with 1.0 mM and the remainder of experiments were carried out at this concentration.

NaCl or NaSCN at a concentration of 100 mM was added to the incubation medium and uptake of

Table 2. Effect of inhibitors on D-glucose uptake

Inhibitor	Concentration	Uptake at 15 sec nM/mg protein (mean ± SEM)			Relative uptake of D-glucose
		D-glucose	L-glucose	Net D-glucose	
Phlorizin	none	0.881 ± 0.022	0.151 ± 0.001	0.730 ± 0.021	1.00
	0.5 mM	0.733 ± 0.061	0.120 ± 0.010	0.613 ± 0.052	0.84 ± 0.04
	1.0 mM	0.674 ± 0.035	0.162 ± 0.016	0.512 ± 0.031	0.70 ± 0.03
Phloretin	none	0.790 ± 0.061	0.200 ± 0.023	0.590 ± 0.063	1.00
	0.05 mM	0.660 ± 0.041	0.130 ± 0.012	0.530 ± 0.042	0.90 ± 0.05
	0.1 mM	0.520 ± 0.070	0.140 ± 0.021	0.380 ± 0.061	0.64 ± 0.05
Fluorodinitrobenzene	none	0.255 ± 0.017	0.117 ± 0.006	0.138 ± 0.008	1.00
	4.0 mM	0.183 ± 0.006	0.111 ± 0.006	0.072 ± 0.003	0.52 ± 0.02

Uptake of D-glucose and L-glucose into vesicles at 15 sec measured as described in Fig. 2. Inhibitors were added to the incubation medium at various concentrations as indicated. Relative net D-glucose uptake is net D-glucose uptake measured with an inhibitor present divided by that measured in the absence of any inhibitor. Values are mean ± SEM of 4 experiments. Vesicles were prepared from different placentas as indicated by the three control values

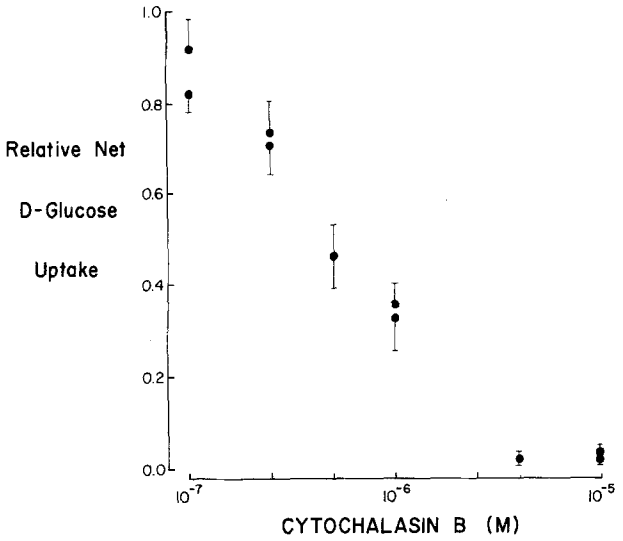

Fig. 3. Effect of cytochalasin B on D-glucose uptake. Uptake of D-glucose and L-glucose at 15 sec measured as described for Fig. 2. Incubation medium and preincubated vesicles contained cytochalasin B in the concentration indicated on the abscissa. In order to include data from a number of vesicle preparations, uptake is expressed as relative net D-glucose uptake as defined in Table 2. Each value is the mean ± SEM of 4 experiments

Table 3. Binding of cytochalasin B to vesicles

Preparation Number	Binding measured in the presence of:	Cytochalasin bound at 5 min (pmol/mg protein)	Percent inhibition
1	L-glucose 80 mM	9.04 ± 0.34	
	D-glucose 80 mM	6.59 ± 0.53	27 ± 1.6
2	L-glucose 80 mM	20.58 ± 1.34	
	D-glucose 80 mM	14.17 ± 0.36	31 ± 1.4

12.5 µl cytochalasin B in Buffer A added to 135 µl placental membrane vesicles in Buffer A to give a final concentration of 0.33 µM [^{3}H] cytochalasin B, and either 80 mM D-glucose or 80 mM L-glucose. Vesicles separated after 5 min by Millipore filtration. Values are mean ± SEM of 4 experiments

sugars measured at 15 sec. The control experiments contained 100 mM KCl or KSCN. Table 1 shows that net D-glucose uptake by the vesicles was not altered under conditions which placed sodium on the outside with none inside the vesicles at the onset of the experiment. This was true for both chloride and for thiocyanate.

Inhibitors in the concentrations indicated were added to the incubation medium and uptake measured at 15 sec. Relative net D-glucose uptake was determined by dividing net D-glucose uptake in the presence of the inhibitor by uptake measured with no inhibitor present. Phloretin inhibited D-glucose uptake at a lower concentration than phlorizin (Table 2). Cytochalasin B was an effective inhibitor of D-glucose transport (Fig. 3).

Table 4. Effect of glucose analogs on D-glucose uptake

Analogs	Concentration (mM)	Relative net uptake of D-glucose at 15 sec
L-glucose	40	1.00
α-methyl-D-glucoside	40	0.96 ± 0.04
2-deoxy-D-glucose	40	0.64 ± 0.07
3-O-methyl-D-glucose	40	0.51 ± 0.14
D-galactose	40	0.81 ± 0.03

Experiments performed as described for Fig. 2, except that uptake stopped at 15 sec. Results expressed as relative net D-glucose uptake as defined in Table 2. Values are mean ± SEM of 4 experiments

In order to distinguish between competitive and noncompetitive inhibition by cytochalasin B we determined the binding of ^{3}H-CB in the presence of either D-glucose or L-glucose. The binding of CB which was present at 0.33 µM was reduced by 30% when the experiments contained 80 mM D-glucose (Table 3). The specificity of facilitated sugar uptake into the placental vesicles was examined by measuring the net uptake of D-glucose (1 mM) in the presence of α-methyl-D-glucoside, 2-deoxy-D-glucose, 3-O-methyl-D-glucose and D-galactose. The D-glucose analogs were present at a final concentration of 40 mM and L-glucose served as the control. The results (Table 4) show that D-glucose uptake is inhibited in the presence of 2-deoxy-D-glucose and 3-O-methyl-D-glucose. D-glucose uptake is decreased to a lesser extent by galactose and uptake measured in the presence of α-methyl-D-glucoside is similar to that with L-glucose in the incubation medium.

Discussion

The results we have reported are qualitatively consistent for those instances where similar mechanisms for glucose transport have been studied in the intact human placenta. Thus, Carstensen et al. (1977) in a perfused preparation found D-glucose transport to be 2.4–2.7 times that of L-glucose, a value similar to what we have observed in the plasma membrane vesicle preparation (*see* Fig. 2). However, given the small size of these vesicles, uptake measured at 15 sec would probably no longer be linear (Hopfer, 1978) so that uptake of D-glucose at the initial rate most likely exceeds that of L-glucose to a much greater extent. These same authors (Carstensen et al., 1977) reported that phloretin in a concentration of 1 mM reduced net D-glucose transport to 0.4 of the control value, which is qualitatively similar to what we have seen (Table 2).

Rice and co-authors (Rice, Rourke & Nesbitt, 1979), also using a perfused placenta, have reported

that substitution of choline chloride for sodium chloride in the perfusate does not affect glucose transport. We have found that with either NaCl or NaSCN outside the placental membrane vesicles and none inside, the uptake of D-glucose is not enhanced. Johnson and Smith (1979) have reported similar results.

The lack of sodium dependence of glucose transport in placental vesicles is in contrast to other tissues. In the presence of a Na^+ gradient the uptake of D-glucose measured at 10–15 sec was 6–14 times that found without the gradient in renal tubule brush-border vesicles (Kinne, Murer, Kinne-Saffran, Thees & Sachs, 1975; Turner & Silverman, 1977). Similarly, in intestinal luminal vesicles sodium gradients caused a twofold increase in glucose uptake with NaCl and a fourfold increase when NaSCN was used to establish the gradient (Murer & Hopfer, 1974). Neither NaCl nor NaSCN gradients had any effect on net D-glucose uptake in our preparation of membrane vesicles from the maternal face of the placental trophoblast. In this respect the placental glucose transport system resembles that of adipose tissue (Carter et al., 1972) where no sodium dependence was observed. Vesicles from basal-lateral membranes of renal tubule cells showed some sodium dependence but the effect was only about half that seen with brush-border vesicles (Kinne et al., 1975).

D-glucose transport systems which are Na^+-dependent are markedly inhibited by phlorizin. Thus, 50% inhibition is seen at 5–10 μM in renal brush-border vesicles (Kinne et al., 1975; Turner & Silverman, 1977) and 50 μM in the intestinal luminal vesicles (Hopfer, Nelson, Perrotto & Isselbacher, 1973). The fact that this level of inhibition is only approached with a phlorizin concentration of 1.0 mM in our placental vesicles is consistent with the lack of Na enhancement of the glucose transport mechanism. The finding that phloretin inhibits D-glucose uptake at a concentration which is less than that of phlorizin shows that the placental vesicle preparation qualitatively resembles the human erythrocyte (LeFevre, 1961).

Cytochalasin B reduced net D-glucose uptake by 50% at a concentration of 0.5 μM (Fig. 3). This concentration is similar to that observed in adipocyte vesicles (Czech, 1976) and erythrocyte ghosts (Taverna & Langdon, 1973; Jung & Rampal, 1977) for an equivalent degree of inhibition. However, the mechanism of CB inhibition is controversial. Based on kinetic measurements in erythrocyte ghosts, Taverna and Langdon (1973) concluded that the inhibition is noncompetitive, while with a similar approach, Jung and Rampal (1977) found competitive interaction. Jung and Rampal (1977) and also Lin and Spudich (1974) reported that 100 mM D-glucose significantly reduced the binding of labeled CB to erythrocyte ghosts. Czech (1976) noted no effect of 100 mM D-glucose on the binding of this inhibitor to adipocyte plasma membranes, while Wardzala, Cushman and Salans (1978) reported inhibition of CB binding to adipocyte membranes by D-glucose concentrations as low as 25 mM. The 30% inhibition of cytochalasin binding which we have observed in the presence of 80 mM D-glucose (Table 3) is equivalent to what has been reported for erythrocyte CB binding measured under similar conditions (Lin & Spudich, 1974, Jung & Rampal, 1977).

The specificity of glucose analogs also allows for comparison with other sugar transport systems. In the placenta we find that the analog with substitution of a methyl for a hydroxyl group at position 1, does not alter the uptake of D-glucose (Table 4). Analogs with substitution at position 2 or 3 have the capability of decreasing the uptake of D-glucose. D-galactose which differs from D-glucose in position 4, interacts with the carrier to a lesser extent than either 2-deoxy-D-glucose or 3-O-methyl-D-glucose. This pattern of specificity is identical to what has been observed for permeability to these four analogs in the erythrocyte (LeFevre, 1961). In contrast, α-methyl-D-glucoside and D-galactose share the D-glucose transporter at the brush-border membrane of the human kidney while 2-deoxy-D-glucose and 3-O-methyl-D-glucose have little if any affinity (Turner & Silverman, 1977).

In summary, these studies show that plasma membranes isolated from the maternal face of human placental trophoblast retain a stereospecific mechanism for glucose transport. The system is not sodium-dependent. It is inhibited by phloretin to a greater extent than by phlorizin and cytochalasin B acts as a potent inhibitor in a competitive manner.

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