Hexose transport in preimplantation rabbit blastocysts

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Summary. Transtrophectodermal 3-O-methyl glucose (3-OMG) transport in the rabbit blastocyst at Days 6 and 7 *post coitum* was investigated to understand better how the trophectoderm can regulate inner cell mass growth by controlling substrate availability. 3-OMG rapidly traversed the trophectoderm and displayed saturation kinetics ($K_m = 4.3 \pm 0.5$ mM, $V_{max} = 79 \pm 3.8$ nmol·cm⁻²). The flux of 3-OMG was inhibited nearly 95% by 10^{-4} M-phloretin, and only 15% by 10^{-4} M-phlorizin. Furthermore, 3-OMG influx was inhibited by cytochalasin B (5 μ M) and was unaffected by removal of sodium. The transport system had a high specificity for 2-deoxy-D-glucose and glucose, and a very low specificity for fructose and 4- α -methyl glucoside. Western blots probed with a polyclonal antibody to the human erythrocyte glucose transport protein and also with a polyclonal antibody to the C-terminus of the glucose transport protein of the rat brain revealed a broad band with a molecular weight of 55 000. Using immunogold labelling techniques, Na⁺-independent glucose transporters were localized to both the apical and basolateral borders of the trophectodermal cell. These results suggest that the mechanism in the trophectoderm responsible for transport of glucose is similar to other sodium-independent glucose transport systems. In addition, 3-OMG influx was unaffected by short-term incubation with progesterone, the progesterone antagonist mifepristone (RU-486), PGF-2 α , PGE-2, insulin, or cAMP. Day-7 *p.c.* embryos also transported hexoses by a similar system because the influx rate and the phlorizin/phloretin sensitivity were the same as in the Day-6 *p.c.* embryo.

Keywords: preimplantation embryo; 3-O-methyl glucose; sugar transport; rabbit

Introduction

The trophectoderm of the preimplantation rabbit blastocyst forms a continuous barrier that separates the outer uterine fluid from the fluid contained within the blastocoele (Hastings & Enders, 1975). As a result, the trophectodermal cells control the environment that bathes the developing embryo. The trophectoderm can therefore be considered to have a role in modulating the growth of the embryo through the regulation of the pH, amino acid content, and the concentrations of other metabolic substrates within the blastocoele.

These solutes most probably traverse the trophectodermal cell by a system of protein channels, carrier molecules, and/or ATP-driven ion pumps. The flux rate of organic molecules via paracellular pathways has been found to be low (Robinson *et al.*, 1989), and this is undoubtedly a consequence of the tight junctions formed between trophectodermal cells (Hasting & Enders, 1975; Benos, 1981). Therefore, regulation of transcellular solute transport systems (i.e. specificity, number, or de-novo synthesis of transporter proteins), can be causally related to growth regulation

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of the embryonic inner cell mass (ICM). For the ICM to have access to glucose, which is utilized by the embryo as an energy source (Fridhandler, 1961), hexose carriers must then exist in the trophoctodermal cells.

In general, glucose can cross the plasma membrane of cells by at least two distinct mechanisms. The first is a sodium-coupled glucose transporter which is found in the apical border of absorptive epithelia (e.g. digestive tract and proximal tubule of the nephron; reviewed by Ullrich, 1979; Esposito, 1984). Glucose transport by this system is inhibited by phlorizin, and, indirectly, by ouabain. In addition, the glucose analogue 4- α methyl glucoside has a high affinity for this system. The second glucose transport system involves a carrier mechanism which operates independently of sodium (Kinne *et al.*, 1975; Gliemann & Rees, 1983). This transport system has been extensively studied in adipose tissue, brain and the human erythrocyte, and is also located on the basolateral membrane of the enterocyte and the proximal tubule cell (Kinne *et al.*, 1975; Esposito, 1984). This transport system is pharmacologically distinct from the sodium coupled system in that cytochalasin B and phloretin are potent, specific inhibitors, while phlorizin is without effect. In addition, the hexose analogue 2-deoxy-D-glucose has a high affinity for this system.

There are a few studies which address the issue of glucose transport in mammalian embryos (Perinchief, 1980; Dabich & Acey, 1982; Gardner & Leese, 1988). However, none of these studies addresses the phenomenon of transtrophoctodermal transport. We used transtrophoctodermal 3-O-methyl glucose flux studies and also polyclonal antibodies raised against either the human erythrocyte glucose transport protein or an artificial peptide homologous to the rat brain glucose transporter C-terminus to characterize and localize the glucose transport system in the rabbit embryo.

Materials and Methods

Collection of blastocysts. New Zealand White rabbits, 6–9 months of age, were mated to fertile bucks. At various times after mating, the females were killed by intravenous injection of 1.5 ml T-61 euthanasia solution (American Hoechst Corp., Somerville, NJ, USA). For embryos at 6 days *post coitum* (*p.c.*; Day 1 = day of mating), the uterine horns were exposed and the blastocysts were flushed from the uteri with Krebs'–Ringer–bicarbonate with glucose (KRBG) consisting of (in mM): NaCl, 119; NaHCO₃, 25; KCl, 4.7; KH₂PO₄, 1.2; MgSO₄, 1.2; CaCl₂, 1.7; D-glucose, 5.5; and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (Hepes), 20; pH adjusted to 7.4. The Day-7 *p.c.* embryos were collected by excising the uteri and gently dissecting the blastocysts out into a Petri dish containing cold KRBG. The blastocysts were then placed in cold (4°C) KRBG until the start of the experiment. Previous studies have indicated that pre-incubation in the cold has no effect on ion or solute transport activity (Robinson *et al.*, 1989).

Influx measurements. [¹⁴C]3-O-methyl glucose (New England Nuclear, Boston, MA, USA; 304.7 mCi/mmol), was diluted to 50 μ Ci/ml in distilled water after first allowing the ethanol/water in which the isotope was packaged to evaporate under an N₂ stream. Trace amounts (0.5–1 μ Ci/ml) were added to 0.5 ml Krebs'–Ringer–bicarbonate without glucose, containing in addition 1 mM unlabelled 3-OMG. After incubation of the blastocyst for an appropriate time, the embryo was removed and washed three times in cold KRB + 1 mM-3-OMG; the long and short axes were measured, and the blastocoele fluid was collected by micropuncture (pipette tip diameters of 5 μ m). Measurement of blastocyst dimensions and fluid collection (described in Robinson *et al.*, 1989) were completed within 3 min of the first wash. Significant loss of tracer from the blastocoele during this wash procedure has not been noted in this study or in a previous study (Robinson *et al.*, 1989). The collected fluid was placed under oil for later analysis. A single volume of the collected blastocoele fluid (up to 10 μ l for the Day-6 *p.c.* embryos and 50 μ l for Day-7 *p.c.* embryos) was measured with a lambda pipette and transferred to a scintillation vial. Then two 10- μ l samples of bathing fluid were collected and placed in scintillation vials for counting, 4 ml Aqueous Counting Scintillant (Amersham Corp., Arlington Heights, IL, USA) were added to each vial, and the samples were counted in a Packard scintillation counter, Model 2000CA.

Immunoelectron microscopy. Day-6 *p.c.* blastocysts were fixed in freshly prepared 2% formaldehyde plus 0.1% glutaraldehyde in 0.1 M-phosphate buffer (pH 7.4) at 4°C for 45 min. After fixation, tissues were washed for 1 h in 0.1 M-NH₄Cl in buffer, dehydrated in a graded series of ethanol (50%, 1 h; 75%, 1 h $\times$ 2) and infiltrated overnight in L.R. White resin (Polysciences, Inc., Warrington, PA, USA) in BEEM capsules (all at 4°C). Tissues were subsequently polymerized in fresh L.R. White resin at 60°C for 24 h. Thin sections were cut with a diamond knife on a Reichert Ultratuc 3 ultramicrotome and collected on bare hexagonal 300-mesh nickel grids.

Immunolabelling was performed at room temperature and the buffer (TBS-BSA) used in all stages consisted of 20 mM-Tris, pH 7.4, 0.5 M-NaCl and 0.1% bovine serum albumin (BSA). Grids were sequentially floated section side down on 30- μ l droplets of the following solutions: 5% normal goat serum in TBS-BSA (30 min), primary antibody

(affinity-purified rabbit anti-human erythrocyte glucose transporter, courtesy of Dr G. Lienhard, Dartmouth Medical School, Hanover, NH, USA) diluted 1:40 in TBS-BSA containing 1% normal goat serum (1.5 h), TBS-BSA (5 × 2 min), secondary antibody (goat anti-rabbit IgG conjugated to 10 nm colloidal gold particles; Janssen Life Sciences, Piscataway, NJ, USA) diluted 1:3 in TBS-BSA containing 1% normal goat serum (1.5 h), TBS-BSA (5 × 2 min), and phosphate-buffered saline (PBS, pH 7.4) (2 × 2 min). Labelled sections were subsequently fixed in 2% glutaraldehyde in PBS (5 min) and washed in distilled water (3 × 2 min). Sections were examined unstained in a JEOL 100 CX transmission electron microscope operating at 60 kV.

Gel electrophoresis and Western blotting. Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a Bio-Rad (Richmond, CA, USA) Mini Protean II slab gel apparatus using the discontinuous buffer system of Laemmli (1970). Rabbit embryos (6 or 7 days *p.c.*; *n* = 5–8) were solubilized in sample buffer containing 2.5% SDS, 13 mM-dithiothreitol, and 50 mM-Tris-HCl (pH 6.8) with 1 mM-phenylmethylsulphonic acid and 1 µg/leupeptin/ml. The embryos were sheared by drawing the sample into a 27-gauge needle. The samples were then run on an 8% gel. In this preparation, there was some difficulty with the glucose transport protein running on the gel in polymeric form. This tendency of the protein to form polymers was decreased by not boiling the sample before loading on the gel. However, some high molecular weight complexes were still apparent (see Figs 6a, b). Electrophoresis was performed at a constant voltage of 100 mV/gel.

Western blot analysis was performed by electrophoretically transferring the protein in the gel to nitrocellulose using a Bio-Rad Mini Trans-Blot Cell (25 mM-Tris, 20% v/v methanol, 192 mM-glycine, pH 8.3 transfer buffer; 100 V constant current for 1 h). Non-specific protein binding sites on the nitrocellulose were blocked by a 1-h exposure to 2% dry non-fat milk in Tris-buffered saline (Tris, 20 mM; NaCl, 500 mM) with 0.05% Tween-20 (TTBS). The nitrocellulose-bound protein was subsequently overlaid with 0.8 µg/ml of the rabbit anti-human erythrocyte glucose transporter or with serum (diluted 1:2500) from a rabbit immunized with an artificial peptide corresponding to the C-terminus of the rat brain glucose transport protein (East Acres Biologicals, Southbridge, MA, USA) (both in TTBS + 1% non-fat milk). Following incubation at room temperature for 2 h, the blots were washed with two TTBS rinses (5 min each), then goat anti-rabbit IgG alkaline phosphatase (Boehringer Mannheim Biochemical, Indianapolis, IN, USA) diluted 1:5000 in TGBS + 1% non-fat milk was added for 1 h. After 2 washes in TTBS, colour development was accomplished with the BCIP/NBT colour development assay kit (Bio-Rad).

Chemicals. Unless otherwise stated, chemicals were obtained from Sigma Chemical Company, St Louis, MO, USA.

Statistics. Comparisons between means were performed with an unpaired *t* test or analysis of variance and the Student–Newman–Keuls test. Data are expressed as mean ± standard error.

Results

Kinetics of 3-OMG transport

The time course of 1 mM 3-OMG uptake was investigated to determine the rate at which this hexose analogue traverses the trophectoderm. Movement of 3-OMG across the trophectoderm was rapid, and the half-time of the uptake was 35.7 ± 1.9 min (Fig. 1). The maximum uptake, as determined from a non-linear curve-fitting program was 63.5 ± 1.7 nmol·cm⁻². Because the velocity of the uptake was linear within the first 10 min, this time point (10 min) was used in all subsequent experiments.

If 3-OMG is transported via a saturable mechanism across the trophectoderm, the uptake of the hexose should display a concentration dependence that is characteristic of Michaelis–Menten kinetics. Results indicate (Fig. 2) that the uptake displays kinetic characteristics that can be described by the Michaelis–Menten equation. The concentration for half-maximum uptake (K_m), as determined by a non-linear curve fitting programme with a diffusion factor included to account for paracellular movement of 3-OMG, was 4.3 ± 0.5 mM, and the calculated V_{max} was 79 ± 3.8 nmol·cm⁻²/10 min.

Transport inhibitors

The dose–response relationships of phlorizin, an inhibitor of Na⁺-dependent hexose transport, and of phloretin, which inhibits Na⁺-independent hexose transport (Esposito, 1984), were examined for Day-6 *p.c.* embryos (Fig. 3). Transport of 3-OMG was inhibited to a much greater extent by phloretin (K_i = 6 µM) than by phlorizin. At 10⁻⁴ M the inhibition of 3-OMG transport by phloretin

Fig. 1. Plot depicting the time-dependence of 1 mM-3-0-methyl glucose uptake in Day-6 *p.c.* rabbit embryos. The error bars are within some symbols ($n = 3-4$ for each point).

Fig. 2. Plot depicting the concentration-dependence of uptake of 3-0-methyl glucose during a 10-min time period. The error bars are within some symbols ($n = 3-4$ for each point).

was nearly complete ($94 \pm 2\%$) whereas phlorizin at this concentration inhibited 3-0MG transport by only $15 \pm 8\%$ ($P < 0.0005$).

Next, $5 \mu\text{M}$ -cytochalasin B, which is an inhibitor of the Na^+ -independent hexose transport system ($K_i = 0.1-0.15 \mu\text{M}$; Gliemann & Rees, 1983; Lavis *et al.*, 1987), was examined for its effect on uptake of 1 mM-3-0MG at 5 and 10 min. The influx (as calculated from the slope of the time-dependent uptake curve) was decreased by this agent from a control value of $1.14 \pm 0.36 \text{ nmol} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$, $n = 8$, to a value of $0.24 \pm 0.07 \text{ nmol} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$, $n = 7$ ($P < 0.025$).

Transport specificity

We next examined the effect of glucose, fructose, and glucose analogues on the transtrophecto-dermal transport of 1 mM-3-0MG as a means of further defining the characteristics of the hexose transporter present in the apical membrane of the blastocyst. Results of these experiments (Fig. 4) indicate that the compound 2-deoxy-D-glucose, which is specific for the Na^+ -independent transport mechanism (Esposito, 1984), was at least as efficacious as glucose in inhibiting 3-0MG transport. 4- α -Methyl-glucoside, which has a high affinity for the Na^+ -dependent system, was only slightly more effective than fructose at inhibiting the 3-0MG flux. 3-0MG was intermediate in its effectiveness.

Fig. 3. Plot depicting the inhibition of the 10-min 3-0-methyl glucose uptake by phloretin and phlorizin in Day-6 *p.c.* rabbit embryos. The error bars are within some symbols ($n = 3$ for each point).

Fig. 4. Graph depicting the efficacy of hexoses and hexose analogues at inhibiting 3-0-methyl glucose uptake in Day-6 *p.c.* rabbit embryos. The error bars are within some symbols ($n = 3$ for each point).

Sodium dependence

Uptake was then assessed in KRB in which the NaCl was replaced with N-methyl-D-glucamine-Cl, and the NaHCO₃ was replaced with choline bicarbonate, thus making the incubation solution Na⁺-free. The control 10 min uptake value of 13.0 ± 0.6 nmol·cm⁻² ($n = 5$) was not different from that measured in Na⁺-free solutions, namely 12.7 ± 0.6 nmol·cm⁻² ($n = 5$).

Hormone effects

Sodium-independent glucose transport proteins can be increased in the plasma membrane by the hormone insulin (Kahn & Cushman, 1986) within 10 min. In addition, the mouse embryo is able to bind insulin at the blastocyst stage (Rosenblum *et al.*, 1986). We investigated whether glucose transport by the rabbit embryo can be increased by maximal (10^{-7} M) concentrations of insulin. Exposure to this concentration of insulin during a 10-min uptake period had no effect on

the flux of 3-OMG (control, $10.6 \pm 0.8 \text{ nmol} \cdot \text{cm}^{-2}$; insulin, $10.0 \pm 0.5 \text{ nmol} \cdot \text{cm}^{-2}$; $N = 5$), for both treatments).

We tested the effect of progesterone at a physiological level (15 ng/ml; Nielsen *et al.*, 1987) on the uptake of 3-OMG to determine whether this pregnancy-associated hormone had any effect on hexose uptake. The experimental protocol embodied the following three conditions: (1) control, 1-h preincubation in KRBG; (2) 1-h preincubation in progesterone, followed by measuring the uptake of 3-OMG in the presence of progesterone; and (3) the same pre-treatment as (2) but with the uptake performed in the absence of progesterone. The results indicate that progesterone at this physiological concentration had no effect on hexose transport (treatment 1, $11.5 \pm 1.1 \text{ nmol} \cdot \text{cm}^{-2}$; treatment 2, $10.6 \pm 0.4 \text{ nmol} \cdot \text{cm}^{-2}$; treatment 3, $10.1 \pm 0.6 \text{ nmol} \cdot \text{cm}^{-2}$; $n = 5$ for each). A 1-h preincubation with 10^{-7} M -mifepristone (a progesterone antagonist) also did not alter the rate of hexose transport in the embryo (control, $8.30 \pm 0.49 \text{ nmol} \cdot \text{cm}^{-2}$; mifepristone, $9.03 \pm 0.28 \text{ nmol} \cdot \text{cm}^{-2}$, $n = 6$, for both), indicating that progesterone, at least on a short-term basis, had no effect on the glucose transport system.

The prostaglandins (PG) E-2 and F-2 α are reported to have an effect on the metabolism of glucose in embryos of other species (Khurana & Wales, 1987b). If these hormones also have metabolic effects in the rabbit embryo, it is possible that these metabolic changes would be accompanied by changes in the rate of hexose transport. Our results demonstrate that there was no effect of PGF-2 α ($4 \times 10^{-6} \text{ M}$; control, 10.9 ± 0.4 , $n = 4$; PGF-2 α , 10.7 ± 0.8 , $n = 4$), or PGE-2 (10^{-6} M ; control 13.7 ± 0.5 , $n = 3$; PGE-2, 12.8 ± 1.2 , $n = 3$), on hexose flux in Day-6 *p.c.* embryos.

Some glucose transport systems are affected by cAMP (Joost *et al.*, 1986). To test whether cAMP has any effect on hexose flux in the rabbit embryo, we examined the change in trans-epithelial transport of 3-OMG in the presence of two concentrations (10^{-5} M , and 10^{-4} M) of forskolin, a compound which is known to elevate intracellular levels of cAMP. Forskolin clearly caused a decrease in 3-OMG flux in a dose dependent manner (Fig. 5). However, a similar result was obtained with 10^{-4} M dideoxyforskolin, an analogue which does not activate adenylate cyclase. Additionally, chlorophenylthio (CPT) cAMP (10^{-4} M) has no effect (Fig. 5). These results indicate that the hexose transport in the rabbit embryo is not modulated by cAMP.

Fig. 5. Effect of forskolin, dideoxyforskolin (ddF), and chlorophenylthio (CPT) cAMP on the 10-min 3-O-methyl glucose uptake in Day-6 *p.c.* rabbit embryos ($n = 4-9$ for each point).

Localization studies

The anti-erythrocyte glucose transporter antibody used in this study was first tested for reactivity in the rabbit embryo by Western blot analysis. Western blotting demonstrates (Fig. 6a) major

bands at molecular masses of 55 000 and 110 000. The band of M_r 55 000 coincides with the molecular mass of known glucose transporters (Matthaei *et al.*, 1987; Blok *et al.*, 1988; Lucas & Zigler, 1988). The band at M_r 110 000 is a dimer of the glucose transport protein. Dimerization of the protein has been observed in other experiments (Lienhard *et al.*, 1984). Under our conditions, we have observed nearly complete dimerization of the protein when the sample is heated before loading on the gel. Although these samples (Fig. 6) were not heated, we suggest that the heat generated in the mini-gel causes dimerization to occur. Because there is a slight possibility that this glucose transport antibody could be reacting with cytoskeletal proteins, we also examined Western blots probed with an antibody generated against the C-terminus portion of the glucose transporter peptide. This blot (Fig. 6b) is identical to Fig. 6(a), indicating specificity of the antibody raised against the erythrocyte glucose transport protein.

Fig. 6. Western blot of solubilized 6 day *p.c.* embryos probed with (a) the anti-erythrocyte glucose transport protein antibody, performed as described in the text and (b) the anti-rat brain glucose transporter C-terminus antibody. Molecular weights of the bands were determined from the position of molecular weight markers. Western blots for Day-7 *p.c.* were similar in appearance.

Immunogold labelling with the rabbit anti-human erythrocyte glucose transporter antibody reveals colloidal gold particles at both the apical and basolateral borders of the trophoblastic cell (Figs 7a, b). This indicates that a similar transport protein is localized to both sides of the

Fig. 7. Immunogold labelling of glucose transport proteins (arrows) in Day-6 *p.c.* trophoctodermal cells: (a) Apical membrane of a trophoctodermal cell; (b) Basolateral membrane of a trophoctodermal cell. Bar = 0.25 μm .

trophoctodermal cell. The basolateral membrane, when qualitatively compared with the apical membrane of the same cell, consistently had a greater colloidal gold particle density.

Transport in 7-day *p.c.* blastocyst

Other transport systems in the trophoctoderm of the rabbit blastocyst are known to change as a function of developmental age (reviewed by Biggers *et al.*, 1988). Phloretin and phlorizin (10^{-4} M) were used as probes to examine the possibility of an alteration in the hexose transport system between Days 6 and 7 *p.c.* The rationale for this approach was that if the control and drug-treated values in the Day-7 *p.c.* embryo are similar to those found in the Day-6 *p.c.* embryo, then it is a

remote possibility that any change occurs in the hexose transport mechanism during this time-frame. Our results indicate that the control value of 3-OMG uptake, and the phloretin/phlorizin (10^{-4} M) sensitivity in the Day-7 *p.c.* embryo (control = 11.0 ± 0.7 nmol·cm $^{-2}$, $n = 4$; phlorizin, 10.1 ± 1.0 nmol·cm $^{-2}$, $n = 7$; phloretin, 1.20 ± 0.15 nmol·cm $^{-2}$, $n = 6$) were similar to those in the Day 6 *p.c.* embryo. In addition, insulin (10^{-7} M) had no effect at this developmental age (not shown).

Discussion

Our results indicate that the Day-6 and -7 *p.c.* rabbit embryo has a glucose transport system that, at the apical and basolateral membrane, operates independently of external Na $^{+}$. The apical Na $^{+}$ -independent transport system in the rabbit blastocyst is similar to the system found in the mouse embryo (Dabich & Acey, 1982; Gardner & Leese, 1988). However, because of the small size of the mouse blastocyst, which precludes transtrophectodermal flux studies, a basolateral Na $^{+}$ -independent glucose transporter was not discovered. To our knowledge, this is the first report which definitively describes a transtrophectodermal hexose transport system.

The Na $^{+}$ -independence of this apical transport system is in contrast to that normally found in epithelia where the apical system is Na $^{+}$ -dependent and the basolateral membrane contains a Na $^{+}$ -independent hexose transport protein. The hexose transport protein in the apical membrane of the trophoctoderm appears to resemble most other Na $^{+}$ -independent glucose transporters in that it has a high specificity for 2-deoxy-D-glucose, and a very low affinity (if any) for 4- α -methyl-glucoside.

Insulin effects on glucose transport have been widely studied in adipose tissues. In rat adipose cells for instance, this hormone causes an increase in the number of glucose transport proteins which are apparently recruited from intracellular pools (Kahn & Cushman, 1986). The half-time of this response is 3–4 min and maximum response is noted at 10 min. Our data reveal no significant change in the 3-OMG flux with the application of 10^{-7} M-insulin for 10 min. We also found no effect at higher doses of insulin (10^{-5} M), or after a 20 min preincubation period (data not shown). This negative result could result from a number of different factors. It may be that there are no insulin receptors on the rabbit embryo, in contrast with the mouse blastocyst (Rosenblum *et al.*, 1986), or perhaps the mechanism whereby insulin increases the number of hexose transporters does not exist.

We speculate that the glucose transporter in the rabbit embryo resembles the erythrocyte/brain-type transporter for the following reasons. The lack of an insulin stimulation and also a cAMP effect on glucose transport, both of which are seen in adipocytes (Karnieli *et al.*, 1981; Joost *et al.*, 1986), makes it improbable that the insulin-responsive protein is present in the embryo. As an additional argument against the presence of an insulin-responsive transporter, immunogold labelling reveals no large intracellular pools of transport protein as was previously seen in unstimulated 3T3-L1 adipocytes (Blok *et al.*, 1988). The glucose transport protein found in the liver is also not a probable candidate because the antibody raised against the C-terminus portion does not cross-react with this peptide (Haspel *et al.*, 1988). Thus, the brain-type glucose transport protein is most probably the hexose carrier in the rabbit blastocyst. The mouse embryo may also have a similar system since it also does not respond to insulin (Gardner & Leese, 1988).

The density of colloidal gold particles at the apical membrane is similar to that found in the adipocyte (Blok *et al.*, 1988). The higher density of colloidal gold particles at the basolateral membrane suggests that the apical membrane, because it has less transport protein, is most likely the rate limiting step in transtrophectodermal hexose transport. However, if the kinetics of hexose transport across the basolateral membrane are very different from those of the apical system, the apical membrane may, in fact, not be rate limiting.

If the basolateral membrane is not the rate-limiting step for transport of glucose across the trophoctodermal cell, then glucose, once it enters the cell, will quickly exit across the basolateral membrane. Glucose then will not be available for metabolism because of the brief time period spent in the trophoctodermal cell. Rapid exit of glucose does appear to occur as preliminary experiments

indicate that the glucose flux into the blastocoele displays a half-time similar to the half-time of the 3-0MG flux (D. H. Robinson & D. J. Benos, unpublished).

Previous studies have shown that PGE-2 and PGF-2 α suppress CO₂ production in mouse embryos (Khurana & Wales, 1987b). To determine whether these hormones have any effect on hexose transport in the rabbit embryo, we examined the effect of exposing the embryos for short periods to PGE-2 or PGF-2 α . Neither prostaglandin tested has an effect on 3-0MG transport. This indicates that these prostaglandins may cause their effect on CO₂ production at a step other than cellular uptake.

Cyclic AMP (cAMP) is known to decrease the $V_{\max}$ and/or the K_m for hexose transport in adipose cells (Joost *et al.*, 1986). The negative result found in the rabbit embryo with CPT cAMP, along with the finding that the forskolin effect is most probably the result of a direct interaction with the hexose transport protein (see below), indicate that the glucose transport system in the rabbit blastocyst is not modulated by any hormones that result in the activation of adenylate cyclase.

Forskolin is known to inhibit glucose transport through the erythrocyte transporter (Sargent & Kim, 1985), and this inhibition is most probably a result of forskolin binding directly to the transport protein (Lavis *et al.*, 1987). The rabbit blastocyst transport system is similar in this sensitivity because both forskolin and dideoxyforskolin (which has no adenylate cyclase-stimulating effect) inhibit 3-0MG flux nearly as well as phloretin, a compound which competitively blocks the transport protein.

The lack of a progesterone effect on the uptake of 3-0MG correlates well with the finding that this hormone has no effect on energy metabolism in the mouse embryo (Khurana & Wales, 1987a). The effect of progesterone found by Perinchief (1980) is most probably a result of the supramaximal concentration of progesterone used.

The flux rate of 3-0MG found in the embryo is in agreement with that found in unstimulated adipose tissue. In isolated rat adipose cells, an uptake of about 0.2 fmol·cell⁻¹·min⁻¹ was found in the presence of 0.1 mM-3-0MG (Karnieli *et al.*, 1981). At this 3-0MG concentration, we found a rate of uptake of 0.14 nmol·cm⁻²·min⁻¹. A previous study found that the surface area of a trophectoderm cell is 3.1×10^{-6} cm²·cell⁻¹ (Daniel, 1964). The 3-0MG uptake, calculated on a cellular basis in the trophectoderm cell, is then about 0.4 fmol·cell⁻¹·min⁻¹. Evidently, this flux, which corresponds to the unstimulated flux in an adipose cell, is sufficient to allow equilibration of hexose between the uterine lumen and the blastocoele fluid.

The function of this system, because it is not affected by hormones, is most probably to allow free passage of hexoses in a constitutive manner across the trophectoderm. Early studies have noted that, in blastocysts, glycolysis predominates over the hexose monophosphate shunt pathway whereas the opposite is true in the morula (Fridhandler, 1961). Perhaps it is necessary for the glucose level in the blastocoele to approach that found in the uterus in order to provide energy substrate for the ICM. In fact, measurements indicate that the glucose concentration within the preimplantation rabbit embryo blastocoele is equal to that in the uterine fluid, namely about 0.4–1 mM (Lutwak-Mann, 1954, 1962).

We conclude that the rabbit blastocyst has a Na⁺-independent glucose transport mechanism located on the apical and basolateral membranes of the trophectoderm. Apparently, this system is constitutively regulated in that the hormones tested have no effect on the transtrophectodermal transport of 3-0-methyl glucose.

Animals used in this study were maintained in accordance with the guidelines of the Animal Use Subcommittee at the University of Alabama at Birmingham and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council [DHEW publication No. (NIH) 85-23, revised 1985]. The University of Alabama at Birmingham has an animal welfare assurance on file with The Office for Protection from Research Risk. The assurance number is A0701 (filed 01/01/86). We thank Mr David Lopez

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