Sodium-gradient-driven, high-affinity, uphill transport of succinate in human placental brush-border membrane vesicles

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Brush-border membrane vesicles isolated from normal human term placentas were shown to accumulate succinate transiently against a concentration gradient, when an inward-directed Na^+ gradient was imposed across the membrane. This uptake was almost totally due to transport into intravesicular space, non-specific binding to the membranes being negligible. The dependence of the initial uptake rate of succinate on Na^+ concentration exhibited sigmoidal kinetics, indicating interaction of more than one Na^+ ion with the carrier system. The Hill coefficient for this ion was calculated to be 2.7. The Na^+ -dependent uptake of succinate was electrogenic, resulting in the transfer of positive charge across the membrane. Kinetic analysis showed that succinate uptake in these vesicles occurred via a single transport system, with an apparent affinity constant of $4.8 \pm 0.2 \mu\text{M}$ and a maximal velocity of $274 \pm 4 \text{ pmol/20 s per mg of protein}$. Uptake of succinate was strongly inhibited by various C_4 or C_5 dicarboxylic acids, whereas monocarboxylic acids, amino acids and glucose showed little or no effect. Li^+ and K^+ could not substitute for Na^+ in the uptake process. Instead, Li^+ was found to have a significant inhibitory effect on the Na^+ -dependent uptake of succinate.

INTRODUCTION

Maintenance of optimal fetal nutrition throughout the fetal development is one of the vital functions of the human placenta. The brush-border membrane of the syncytiotrophoblast, which is in direct contact with the maternal blood, possesses specific transport systems for several nutrients. These transport systems participate in the transfer of nutrients from the mother to the fetus for utilization in the fetal metabolism. In addition, they also provide nutrients for the maintenance of optimal placental growth and development.

Until now, investigations have been primarily focused on characterizing the placental transfer of the major nutrients glucose and amino acids (Johnson & Smith, 1980; Bissonnette, 1982; Ingermann *et al.*, 1983; Yudilevich & Sweiry, 1985). In recent years, however, there has been a surge of interest in the placental transfer of other nutrients as well. By using brush-border membranes isolated from human term placentas, transport systems specific for dipeptides (Ganapathy *et al.*, 1985), vitamins (Friedman *et al.*, 1977; Green & Ford, 1984; Ingermann *et al.*, 1986) and many inorganic ions such as Na^+ (Balkovetz *et al.*, 1986), iron (Wada *et al.*, 1979; Seligman *et al.*, 1979), sulphate (Cole, 1984), chloride (Shennan *et al.*, 1986) and phosphate (Brunett & Allard, 1985) have been described in these membranes. However, very little is known about the contribution made to the energy needs of the fetus and of the placenta by various metabolic intermediates that are present in the maternal circulation. Of particular interest are the intermediates of glycolysis and the tricarboxylic acid cycle, which occur in significant quantities in the maternal blood. The present investigation was undertaken to examine if the human placental brush-border membranes possess specific transport systems for these intermediates. In this study,

we characterized the uptake of radiolabelled succinate into brush-border membrane vesicles isolated from human term placentas. The data provide evidence for the first time that these membranes contain a high-affinity, Na^+ -dependent, active transport system for succinate. Competition experiments revealed that this transport system is specific for dicarboxylic acids, many of which are tricarboxylic acid-cycle intermediates, and it has little or no affinity for glucose, amino acids and monocarboxylic acids.

EXPERIMENTAL

Materials

[2,3- ^{14}C]Succinic acid (sp. radioactivity 47.8 mCi/mmol) was obtained from New England Nuclear, Boston, MA, U.S.A. All other chemicals were of the highest purity available and were obtained either from Sigma, St. Louis, MO, U.S.A., or from U.S. Biochemical Corp., Cleveland, OH, U.S.A.

Methods

The syncytiotrophoblast brush-border membrane vesicles were prepared from normal human term placentas by using the Mg^{2+} -precipitation technique as described previously (Ganapathy *et al.*, 1985; Balkovetz *et al.*, 1986). The purity of the membrane preparations was monitored by measuring the activity of alkaline phosphatase (Ganapathy *et al.*, 1986). In most experiments, the membrane vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 300 mM-mannitol. In experiments where the effect of a K^+ -diffusion potential on the uptake was studied, the vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 100 mM- K_2SO_4 . The membrane suspension

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(protein concn. 6 mg/ml) was distributed in small portions in plastic tubes and stored in liquid nitrogen until use.

Uptake measurements were made by a rapid filtration method as described previously (Ganapathy *et al.*, 1981), with Millipore filters (DAWP type, pore size 0.65 μ m). Uptake was initiated by mixing 40 μ l of membrane suspension with 160 μ l of uptake buffer containing radiolabelled succinate. The composition of the uptake buffer in most experiments was 10 mM-Hepes/Tris, 100 mM-mannitol, 100 mM-NaCl, pH 7.5. The uptake was terminated by adding 3 ml of ice-cold stop buffer (5 mM-Hepes/Tris, 155 mM-NaCl, pH 7.5), and the mixture was filtered. The filter was washed with 3 $\times$ 5 ml of the stop buffer, and the radioactivity associated with the filter was then counted.

The experiments were regularly done in duplicate or triplicate, and the variation was always less than $\pm 10\%$ of the mean value.

RESULTS

Effect of an inward-directed Na^+ gradient on succinate uptake

Uptake of 10 μ M-succinate into the placental brush-border membrane vesicles was studied in the presence of an inward-directed Na^+ gradient or a K^+ gradient (Fig. 1). When a Na^+ gradient was present across the membrane ($[\text{Na}^+]_o = 80$ mM; $[\text{Na}^+]_i = 0$), uptake of succinate was rapid and the solute was transiently accumulated inside the vesicles against a concentration gradient. The intravesicular concentration of succinate measured with a 5 min incubation was 7-fold greater than the equilibrium value (60 min incubation). On the contrary, uptake in the presence of a K^+ gradient ($[\text{K}^+]_o = 80$ mM; $[\text{K}^+]_i = 0$) was very slow, and there was no indication of accumulation of the solute against a concentration gradient. Uptake of succinate showed an absolute requirement for Na^+ , because the initial uptake rate in the presence of Na^+ was at least 150-fold greater than in the presence of K^+ .

To determine whether the uptake of succinate measured in placental brush-border membrane vesicles was due to transport into the intravesicular space or binding to the membranes, we studied the effect of osmolality of the incubation medium on the equilibrium uptake of succinate. If the uptake represented transport into the intravesicular space, the equilibrium concentration of succinate inside the vesicles would vary with the osmolality of the incubation medium. It was found that the equilibrium uptake of succinate decreased as the osmolality of the incubation medium increased, and the plot of equilibrium uptake versus $1/\text{osmolality}$ was linear and passed through the origin (results not shown). These results show that the uptake of succinate measured in these vesicles was exclusively due to transport into the intravesicular space, and binding to the membranes, if any, was negligible.

The dependence of the initial rate of succinate uptake (15 s incubation) on the concentration of extravesicular Na^+ (intravesicular Na^+ concentration was zero) was then investigated. The uptake rate increased with increasing concentration of Na^+ (Fig. 2). The sigmoidal shape of the curve in Fig. 2 indicated that the carrier system responsible for the uptake of succinate interacts

Fig. 1. Uptake of succinate in the presence of a Na^+ gradient or a K^+ gradient

The membrane vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 300 mM-mannitol. Uptake of 10 μ M-succinate was measured in 10 mM-Hepes/Tris buffer, pH 7.5, containing 100 mM-mannitol and either 100 mM-NaCl (●) or 100 mM-KCl (○).

Fig. 2. Dependence of succinate uptake on Na^+ concentration

The membrane vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 300 mM-mannitol. Uptake of 10 μ M-succinate was measured with a 15 s incubation in 10 mM-Hepes/Tris buffer, pH 7.5, containing different concentrations of NaCl. The concentration of mannitol in the uptake buffer was adjusted to maintain osmolality.

Fig. 3. Hill plots describing the relationship between the initial rate of succinate uptake and the Na^+ concentration

The data in Fig. 2 were used to construct the plots. The initial uptake rate (v) was plotted against $v/[\text{Na}^+]^n$, and n was assigned a value of 1 (●), 2 (○) or 3 (■). Units: v , pmol/15 s per mg; $[\text{Na}^+]$, mM.

with more than one Na^+ ion. In order to determine the number of Na^+ ions interacting with the carrier, Hill plots (v versus $v/[\text{Na}^+]^n$) were constructed, assigning n a value of 1, 2 or 3. Fig. 3 shows that when $n = 1$ or 2, the plots are curvilinear. But the plot is close to a straight line ($r = -0.988$) when $n = 3$. The exact value of the Hill coefficient for the experimental data in Fig. 2 was found to be 2.7 ($r = -0.999$). These results indicate that the succinate-transport system interacts with 3 Na^+ ions.

Effect of a membrane potential on succinate uptake

Succinic acid exists predominantly as a dicarboxylate ion with two negative charges at pH 7.5. Since the succinate: Na^+ stoichiometry appears to be 1:3, succinate uptake should result in transfer of positive charge across the membrane. Therefore the Na^+ -dependent succinate uptake is expected to be potential-sensitive. The effect of membrane potential on succinate uptake was investigated by using two different approaches. First, the influence of a valinomycin-induced inside-negative K^+ -diffusion potential on the initial rate of succinate uptake was examined. Uptake of 10 μM -succinate was measured with 15 s incubations in the presence of an outward-directed K^+ gradient ($[\text{K}^+]_i = 200 \text{ mM}$; $[\text{K}^+]_o = 40 \text{ mM}$) and an inward-directed Na^+ gradient ($[\text{Na}^+]_o = 160 \text{ mM}$; $[\text{Na}^+]_i = 0$). Under these conditions, addition of valinomycin, a K^+ ionophore, would enhance the diffusion of K^+ down its gradient and thus generate an inside-negative potential. Such treatment significantly increased succinate uptake ($P < 0.01$) compared with the uptake measured in the absence of valinomycin (Table 1). In the second approach, we studied the influence of membrane potential induced by diffusion of different anions on the initial rate of Na^+ -dependent uptake of succinate. In these experiments, membrane vesicles preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 300 mM-mannitol were used. Uptake was measured in the presence of 50 mM- Na_2SO_4 , 100 mM- NaCl or 100 mM- NaNO_3 (mannitol was added to adjust for osmolality). The diffusion coefficients of the anions are in the following order: $\text{NO}_3^- > \text{Cl}^- > \text{SO}_4^{2-}$. Table 1 shows that the uptake of succinate was manyfold greater in the

Table 1. Effect of membrane potential on succinate uptake

For the K^+ -diffusion potential, membrane vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 100 mM- K_2SO_4 . Uptake of 10 μM -succinate was measured with a 15 s incubation in 10 mM-Hepes/Tris buffer, pH 7.5, containing 100 mM- Na_2SO_4 . A stock solution of valinomycin made in ethanol was added to the uptake buffer to give a final concentration of 10 μM of the ionophore during uptake experiment. An equal amount of ethanol was included in control experiments. For the anion-diffusion potential, membrane vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 300 mM-mannitol. Uptake of 10 μM -succinate was measured with a 15 s incubation in 10 mM-Hepes/Tris buffer, pH 7.5, containing 50 mM- Na_2SO_4 plus 150 mM-mannitol, 100 mM- NaCl plus 100 mM-mannitol, or 10 mM- NaNO_3 plus 100 mM-mannitol. The results are given as means $\pm$ S.D. for three determinations.

Diffusion potential	Experimental conditions	Succinate uptake (pmol/15 s per mg)
K^+	Control	24.0 ± 0.6
	Valinomycin	31.5 ± 0.1
Anion	Sulphate	44.7 ± 1.3
	Chloride	161.1 ± 2.5
	Nitrate	180.4 ± 2.7

presence of Cl^- and NO_3^- than in the presence of SO_4^{2-} . These data demonstrate that the Na^+ -dependent succinate uptake is an electrogenic process and provide support for a succinate: Na^+ stoichiometry of 1:3.

Kinetics of succinate uptake

The dependence of the initial rate of succinate uptake on the concentration of succinate was then studied. The concentration range employed was 1.25–50 μM . Initial uptake rates were measured with a 20 s incubation, and all measurements were done in the presence of an inward-directed Na^+ gradient ($[\text{Na}^+]_o = 120 \text{ mM}$; $[\text{Na}^+]_i = 0$). Fig. 4 shows that the uptake of succinate into the placental brush-border membrane vesicles occurred via a saturable process. The Eadie-Hofstee plot (initial velocity/substrate concentration versus initial velocity) was linear ($r = -0.997$) (Fig. 4, insert), providing evidence for the presence of a single transport system for succinate uptake in these membranes. The kinetic constants (affinity constant, K_t , and maximal velocity, V_{max}) were calculated by linear-regression analysis of the data from the Eadie-Hofstee plot. The apparent affinity constant was $4.8 \pm 0.2 \mu\text{M}$ (mean $\pm$ S.D.) and the maximal velocity was $274 \pm 4 \text{ pmol/20 s per mg of protein}$.

Substrate specificity of the succinate-uptake system

Competition experiments were done to determine the substrate specificity of the succinate-uptake system in placental brush-border membrane vesicles. We examined the effect of various unlabelled compounds (glucose, amino acids and carboxylic acids) on the uptake of radiolabelled succinate. The data are given in Table 2. Unlabelled succinate strongly competed with labelled succinate for the uptake process: at 2 mM, unlabelled succinate totally abolished the uptake of 10 μM radiolabelled succinate. Malate, oxalomalate, fumarate,

Fig. 4. Relationship between initial uptake rate and succinate concentration

The membrane vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 300 mM-mannitol. Uptake of succinate was measured with a 20 s incubation in 10 mM-Hepes/Tris buffer, pH 7.5, containing 150 mM-NaCl. The concentration of succinate was varied between 1.25 and 50 μ M. Insert: Eadie-Hofstee plot (initial velocity/succinate concentration versus initial velocity).

Table 2. Effects of various compounds on succinate uptake in placental brush-border membrane vesicles

Membrane vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 300 mM-mannitol. Initial rates (15 s incubation) of succinate uptake were measured in 10 mM-Hepes/Tris buffer, pH 7.5, containing 100 mM-NaCl and 100 mM-mannitol. Concentration of labelled succinate was 10 μ M and of inhibitors was 2 mM. Results are given as means $\pm$ S.D. for three determinations.

Inhibitor	Succinate uptake	
	(pmol/15 s per mg)	(%)
None	158.7 $\pm$ 7.3	100
Succinate	0	0
DL-Malate	10.3 $\pm$ 0.6	7
Oxalomalate	19.3 $\pm$ 0.8	12
Fumarate	3.9 $\pm$ 0.2	3
Glutarate	8.2 $\pm$ 0.3	5
α -Oxoglutarate	4.6 $\pm$ 0.1	3
Citrate	41.2 $\pm$ 0.7	26
Oxalate	141.4 $\pm$ 3.3	89
Maleate	156.2 $\pm$ 2.7	98
Malonate	147.0 $\pm$ 2.1	93
Lactate	144.2 $\pm$ 1.5	91
Pyruvate	154.8 $\pm$ 3.6	98
Aspartate	114.5 $\pm$ 0.9	72
Glutamate	157.9 $\pm$ 3.2	100
Alanine	157.5 $\pm$ 3.8	99
Glucose	162.5 $\pm$ 2.6	102

glutarate and α -oxoglutarate were equally potent in inhibiting the uptake of succinate. Citrate and aspartate also effectively competed with succinate for the uptake process. On the other hand, oxalate, maleate and

Fig. 5. Effects of Li⁺ on Na⁺-dependent succinate uptake

The membrane vesicles were preloaded with 10 mM-Hepes/Tris buffer, pH 7.5, containing 300 mM-mannitol. Uptake of 10 μ M-succinate was measured with a 15 s incubation in 10 mM-Hepes/Tris buffer, pH 7.5, containing 100 mM-NaCl and different concentrations of KCl and LiCl. The combined concentration of KCl plus LiCl was maintained at 50 mM.

malonate failed to inhibit succinate uptake. Similarly, the monocarboxylic acids pyruvate and lactate, the amino acids glutamate and alanine, and glucose had no effect on succinate uptake.

Effect of Li⁺ on the Na⁺-dependent succinate uptake

When we examined the specificity of univalent cations to stimulate succinate uptake, only Na⁺ showed the stimulatory effect, and other ions such as K⁺ and Li⁺ failed to substitute for Na⁺ (results not shown). During the course of this work, we noticed that Li⁺ not only failed to enhance succinate uptake on its own, but also inhibited the Na⁺-stimulated succinate uptake. Fig. 5 describes the effect of increasing concentration of Li⁺ on Na⁺-dependent succinate uptake. A significant inhibition ($P < 0.05$) was observed at a Li⁺ concentration as low as 1 mM. At 10 mM, Li⁺ inhibited Na⁺-dependent succinate uptake by approx. 50%.

DISCUSSION

The presence of a Na⁺-dependent active transport system for succinate and other tricarboxylic acid-cycle intermediates has been demonstrated in brush-border membrane vesicles isolated from renal cortex (Kippen *et al.*, 1979). The characteristics of this transport system, including substrate specificity, kinetics, cation-dependence, succinate:Na⁺ stoichiometry and electrogenicity, have all been worked out in detail (Wright, 1985).

Studies on the transport of dicarboxylic acids across the basal-lateral membrane of renal tubular cells are limited, but there is indication that a Na^+ -dependent transport system for dicarboxylates is present in these membranes also (Burckhardt, 1984; Fritzsche *et al.*, 1984; Ullrich *et al.*, 1984, 1987). There have been only three studies on the transport of succinate across the brush-border membrane of small intestine, and the results of these studies suggest that the transport system may be present in the intestinal brush-border membrane in some, but not all, species. Rabbit small-intestinal brush-border membranes do not possess a carrier system for succinate and other tricarboxylic acid-cycle intermediates (Stevens *et al.*, 1982; Schell *et al.*, 1983), whereas there is evidence that these solutes are transported across hamster small-intestinal brush-border membrane (Browne *et al.*, 1978).

Until now, there has been no indication that the transport system specific for tricarboxylic acid-cycle intermediates exists in any other cell plasma membrane. In this paper, we provide evidence for the first time for the presence of a Na^+ -gradient-driven, high-affinity, active transport system for succinate in the human placental brush-border membrane vesicles. In these vesicles, succinate was taken up into an osmotically responsive intravesicular space, and non-specific binding, if any, was negligible. The uptake system showed almost an absolute requirement for Na^+ . Concentrative uptake of succinate into these vesicles could be demonstrated when an inward-directed Na^+ gradient was imposed across the membrane. The uptake occurred via a single transport system with a very high affinity for succinate ($K_t = 4.8 \pm 0.2 \mu\text{M}$).

The properties of the succinate-uptake system in human placental brush-border membrane vesicles are similar to those in renal brush-border membrane vesicles. Uptake of succinate in renal brush-border membrane vesicles is Na^+ -dependent (Wright *et al.*, 1980; Nord *et al.*, 1982; Fukuhara & Turner, 1983), electrogenic (Wright *et al.*, 1981; Kragh-Hansen *et al.*, 1982a,b; Sheikh *et al.*, 1982; Jorgensen *et al.*, 1983; Schell *et al.*, 1983; Fukuhara & Turner, 1983; Schell & Wright, 1985) and mediated by a single transport system (Wright *et al.*, 1980; Nord *et al.*, 1982; Fukuhara & Turner, 1983). The data in the present paper show that succinate uptake in human placental brush-border membrane vesicles is also Na^+ -dependent. The requirement for Na^+ is absolute, because other univalent cations failed to enhance succinate uptake. Studies on the effects of ionophore-induced and anion-induced membrane potentials on succinate uptake suggest that the uptake process is electrogenic, resulting in a transfer of positive charge across the membrane. Kinetic analysis shows that succinate uptake in these membrane vesicles occurs via a single carrier system.

A notable difference, however, is in the affinity constants of the transport systems in placenta and kidney. In renal brush-border membranes, the apparent affinity constant for succinate has been calculated under variety of conditions, and the value has ranged between 0.11 mM and 1 mM, depending on the influx-measurement conditions (Wright *et al.*, 1980; Nord *et al.*, 1982; Fukuhara & Turner, 1983). K_t values calculated in voltage-clamped membrane vesicles were significantly higher than the values in control vesicles. This is because changes in membrane potential across the membrane have profound effects on the affinity constant of the

uptake system (Wright *et al.*, 1983). In the present study, we have calculated the apparent affinity constant for succinate uptake in placental brush-border membrane vesicles, using zero-*trans* conditions (i.e. at the beginning of the uptake, Na^+ and succinate were present only on the external side of the vesicles and the intravesicular concentration of these substances was zero) and a Na^+ concentration of 120 mM. The apparent affinity constant was found to be $4.8 \pm 0.2 \mu\text{M}$. These experiments were conducted in membrane vesicles in which membrane potentials were not controlled. However, the affinity constant calculated under similar conditions in renal brush-border membrane vesicles was 0.11 mM (Wright *et al.*, 1980), which is at least 20 times the value in the human placenta. Thus the placental transport system exhibits manifold greater affinity for succinate than does the renal transport system.

Although there is consensus on the electrogenic nature of the Na^+ -succinate uptake in renal brush-border membrane vesicles, controversy exists on the Na^+ :succinate stoichiometry. S. H. Wright *et al.* (1982) and E. M. Wright *et al.* (1984), who demonstrated interaction of approx. 3 Na^+ ions with the succinate-uptake system, proposed that succinate is transported as a dicarboxylate and that the Na^+ :succinate²⁻ stoichiometry is 3:1. On the other hand, Fukuhara & Turner (1983), who obtained a coupling of 2 Na^+ ions with the uptake system, concluded that succinate is transported as a monocarboxylate and that the Na^+ :succinate⁻ stoichiometry is 2:1. Our results in human placental brush-border membrane vesicles show that the succinate-uptake system in these membranes interacts with 3 Na^+ ions, and thus agree with the work of Wright and his co-workers just cited, but disagree with that of Fukuhara & Turner (1983). The electrogenic nature of the Na^+ -succinate co-transport in placental membrane vesicles, and the fact that succinate exists predominantly as a dicarboxylate ion under the experimental conditions, therefore suggest that succinate is transported as a dicarboxylate ion in human placenta and that the Na^+ :succinate²⁻ stoichiometry is 3:1.

The substrate specificities of the succinate transport system in placenta and kidney are similar. The placental transport system, like its renal counterpart, is specific for C_4 or C_5 dicarboxylic acids. Citrate, a tricarboxylic acid, is an effective inhibitor of succinate uptake, but it is likely that the dicarboxylate rather than the tricarboxylate ion is the active species. Since fumarate is a potent inhibitor of succinate uptake, whereas maleate is not, the transport system is stereospecific, requiring the carboxyl groups to be in a *trans*-configuration. Oxalate, a C_2 dicarboxylic acid, and malonate, a C_3 dicarboxylic acid, failed to interact with the succinate-transport system. The monocarboxylic acids pyruvate and lactate also had no inhibitory effect on succinate uptake. The transport system exhibits little or no affinity for glucose and amino acids.

Another striking similarity between the placental and renal brush-border succinate-transport systems is their susceptibility to inhibition by Li^+ . Like in kidney (E. M. Wright *et al.*, 1982), Li^+ could not substitute for Na^+ in enhancing succinate transport in placenta, and it also inhibits the Na^+ -dependent uptake of succinate. Similar results with Li^+ have been obtained for dicarboxylate transport in renal basal-lateral membrane vesicles (Burckhardt, 1984).

The physiological role of the Na⁺-dependent dicarboxylate-transport system in renal brush-border membranes is to ensure efficient re-absorption of the tricarboxylic acid-cycle intermediates from the glomerular filtrate. Since the kidney can utilize these compounds as a source of metabolic energy, the dicarboxylate transport systems of brush-border as well as basal-lateral membranes may function in providing this organ with adequate metabolic fuel. The succinate-transport system of the human placenta may play a similar role in providing the tricarboxylic acid-cycle intermediates to be utilized in placental and fetal metabolism. These intermediates circulate in maternal blood in significant quantities. The normal plasma concentrations of succinate (Krebs, 1950), α -oxoglutarate (Krebs, 1938) and citrate (Hodgkinson, 1963), for example, have been reported to be approx. 40 μ M, 50 μ M and 130 μ M respectively. Therefore human placenta can extract these compounds from the maternal blood via the Na⁺-gradient-driven, high-affinity, dicarboxylate-transport system localized in the placental brush-border membrane.

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