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NUTRIENT TRANSPORT PATHWAYS ACROSS THE EPITHELIUM OF THE PLACENTA

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INTRODUCTION

Fetal growth and development depends on a continuous nutrient supply from maternal blood (9, 103). In the human placenta the supply process includes uptake from maternal blood and transfer across the syncytiotrophoblast and cytotrophoblast layers, the underlying basal lamina, the fetal connective tissue space, and the fetal capillary endothelium. Although paracellular mechanisms exist (85, 132), the quantitative and qualitative importance of these pathways to overall nutrient flux is yet to be determined. Flux across the syncytiotrophoblast and its plasma membranes represents the rate-limiting steps in maternal-fetal transfer of most important nutrients. Most sites of known mechanisms of cellular transport are localized to the maternal- and fetal-facing plasma membrane surfaces of the syncytiotrophoblast (124).

The placental syncytiotrophoblast is a continuous epithelial layer. It covers the maternal surface of the human placenta (124, 154) and forms by fusion and terminal differentiation of the underlying cytotrophoblast. The syncytiotrophoblast is a polarized epithelium that resembles the epithelia of kidney and intestine, except that lateral cell borders are absent and the epithelium forms a multinucleated syncytium. The microvillous membrane of the syncytiotrophoblast is in contact with the maternal blood while the basal membrane faces the fetal circulation. These two membranes can be isolated from the same placenta by well-established procedures of mild shearing and centrifugation (12, 82) or by a newer method using homogenization (65). Alkaline phosphatase and 5' nucleotidase are localized to the microvillous membrane, which obtains its "finger-like" structure from highly ordered actin-containing microfilaments (13, 84). The basal membrane contains the sodium potassium ATPase and β-adrenergic receptor coupled to adenyl cyclase (82).

Regulation of syncytiotrophoblast transport mechanisms is potentially of great importance in the control of fetal nutrient supply. Regulation of transport may be affected either by intrinsic mechanisms, in which substrate or cellular proteins interact with transporters, or by extracellular or circulating hormones or effectors (83, 104, 125, 153, 155, 157) and their receptors in the trophoblast (11, 21, 106). In addition to physiologic regulators, considerable evidence suggests that environmental substances such as ethanol and cannabinoids can alter transport of various nutrients (47, 113). The two trophoblast membranes can communicate across the syncytial cytoplasm by cyclic AMP generation at the basal membrane in response to stimuli originating in the fetus, which in turn stimulates a protein kinase in the microvillous membrane (1). This and other communication pathways involving other receptors and protein kinases at the two membranes provide potential mechanisms by which stimuli originating in the fetus or the mother can regulate transport of substrates across the maternal- or fetal-facing membranes.

The mechanisms of nutrient transfer from mother to fetus have been investigated by a variety of methods. These include in vivo, chronic maternal and fetal blood sampling in sheep and other animals (9, 103), perfusion of the delivered human placenta or its cotyledons (116, 136), in vitro incubation of placental tissue fragments (128), isolation and incubation of maternal- and fetal-facing plasma membranes from human placenta (12, 65, 82), and, most recently, investigations in our and other laboratories using cultured human trophoblast. Each of these methods can potentially contribute important and ultimately complementary data on the mechanisms of placental solute transfer. This review summarizes investigations of the cellular and membrane mechanisms underlying the placental transport of a variety of important nutrients.

ORGANIC NUTRIENTS

Monosaccharides

Glucose is a major fetal and placental fuel whose metabolism accounts for a substantial fraction of fetal oxygen consumption (9, 103). The stereospecificity and saturability of transplacental glucose transfer indicates that it is a mediated process. Transfer mechanisms mediating the facilitated diffusion of glucose have been characterized in microvillous and basal plasma membranes in our own and other laboratories (67, 71, 73). The transporters interact strongly with D-aldohexoses and other sugars that can assume the C-1 chair configuration. The placental microvillous and basal membrane carriers differ from those of the apical surface of intestine and kidney, which possess sodium-dependent concentrative glucose transporters. Thus, while kidney and intestine may concentrate glucose against a concentration gradient, the placental syncytiotrophoblast lacks such ability. Some (but not all) studies have reported regulation of microvillous membrane glucose uptake by insulin under certain conditions and at concentrations (10^{-9}M) somewhat above the high physiologic range (24, 71).

Cytochalasin B-labelling in two laboratories has identified the microvillous membrane transporter as a protein of approximately 50 kDa (68, 72). More recent molecular studies have suggested that the human placenta contains either the isoform GLUT 1 or a mixture of GLUT 1 and GLUT 3 (10). K_m values for placental glucose transporters have been reported as 25 mM (71, 73) and 3–5 mM (67), a range more in keeping with the K_m of GLUT 1 and GLUT 3. The higher K_m values were found in studies using equilibrium exchange, a procedure that may more closely parallel in vivo conditions and that in general results in higher K_m and V_{max} values than a zero trans procedure. Indeed the lack of saturation of the overall maternal-fetal transfer process at glucose concentrations severalfold higher than in maternal blood (66) is in agreement with the higher range of K_m values.

The glucose transporters of the two plasma membranes supply glucose for fetal and placental metabolism under conditions in which the glucose concentration in the fetal circulation is approximately 70% of that in the maternal circulation (9, 103). Using kinetic parameters and surface area determinations, an investigation has estimated that the glucose transfer capacity of the microvillous membrane is manyfold higher than would be required to supply fetal and placental needs for glucose (71). Thus, in vivo the trophoblast cytosol is likely to be nearly equilibrated with maternal plasma glucose. The maternal-fetal glucose gradient may arise from limitation of overall transsyncytial transfer by the lower transport capacity of the basal membrane with its smaller surface area (73).

Amino Acids

The transfer of amino acids across the syncytiotrophoblast involves mediated transport mechanisms at the microvillous and basal membrane and possibly diffusion. Analysis of the process is complex and incomplete because of the large number of amino acids, the overlapping specificity of amino acid transport mechanisms, and the utilization of amino acids for nutrition of the trophoblast itself. Plasma membrane transport systems have been the subject of a number of recent reviews that describe their specificity, sodium-dependence, and other important properties (31, 147).

NEUTRAL AMINO ACIDS The microvillous membrane of placenta utilizes transport systems common to many cell types. This is in contrast to intestinal or renal brush borders, which utilize specialized neutral amino acid transport systems (131) that may be similar to system $B^{0,+}$ described in developing mouse blastocysts (147). Reported placental systems include (*a*) system A—a sodium-dependent transporter that interacts strongly with alanine, serine, methylaminoisobutyric acid (MeAIB), and proline and is a ubiquitous concentrative transporter of amino acids; (*b*) system N—a sodium-dependent system that interacts strongly with histidine and glutamine; and (*c*) various sodium-independent systems (17, 74, 79). One of the sodium-independent transporters interacts strongly with leucine, tryptophan, tyrosine, and phenylalanine and resembles system L (52, 88). A second sodium-independent system apparently reacts strongly with alanine and serine and weakly with the branched-chain and aromatic amino acids (74). System T and system ASC have been reported to be absent in microvillous membrane (52, 74). Recent evidence, however, suggests that storage conditions of the membrane vesicles may influence the transport systems subsequently identified (78).

The transport systems of placental basal membrane resemble those of the basolateral membranes of intestine that possess sodium-dependent systems similar to the classical A and ASC systems as well as a sodium-independent L

system (99). At least three mediated processes are present: (*a*) a sodium-dependent A-like system shared by MeAIB that is similar to the system employed by microvillous membrane; (*b*) a second sodium-dependent ASC-like system that interacts with alanine, serine, and apparently cysteine but is resistant to MeAIB; (*c*) a sodium-independent system that interacts with leucine, phenylalanine, and BCH and resembles system L (61) and potentially another sodium-independent system that transports tyrosine (88). To our knowledge, basal membrane has not been investigated for system N. Some rather ambitious investigations attempted to elucidate a broad scope of transporters on either membrane using a small number of inhibitors employed at high concentrations (87, 89). The potential for noncompetitive inhibition and system overlap under these conditions makes these studies difficult to interpret.

Transporters in the two membranes must act in concert to bring about the concentrative transfer of amino acids from mother to fetus. The physiological sodium gradient drives the sodium-dependent systems of the two plasma membranes toward uptake of amino acids from maternal and fetal circulations into the syncytium. With the larger surface area of the microvillous membrane (140) and its higher system A transport activity, the A system of microvillous membrane is likely to be responsible for generating the high syncytial concentration of alanine (74) (estimated as 7-fold greater than the concentration in maternal blood and 4-fold greater than the concentration in fetal blood) (108).

Transport from the trophoblast to the fetus across the basal membrane may occur via both system L and nonmediated pathways (61). The roles of the sodium-dependent systems in basal membrane are not established. They may, as suggested for similar systems in the intestine (99), provide mechanisms for trophoblast nutrition or for amino acids to leave the fetus under some metabolic conditions.

Cellular regulation of amino acid transport must involve regulation of transport systems of either of the two surface membranes. System A is known to be regulated by two mechanisms in placental tissue. One requires protein synthesis and provides a threefold or greater increase in V_{max} (127), and the other, transinhibition, is a feedback process by which intracellular substrate can inhibit uptake (129). The high trophoblast concentration of A system substrates established by microvillous membrane uptake could reduce uptake by system A in basal membrane. Both of these regulatory mechanisms are specific to system A and do not affect system L or system ASC. Transinhibition may serve to maintain a constancy of trophoblast amino acid concentrations with variation in maternal metabolic environment. Insulin, another regulator, is known to increase system A activity in muscle. Insulin is apparently without effect in placental villous tissue (128), although recent

data from trophoblast primary cultures suggests that insulin at a concentration (10^{-9}M) slightly above the high normal range increases the V_{max} of aminoisobutyrate uptake (Peter Karl and Stanley Fisher, personal communication).

ANIONIC AMINO ACIDS Anionic amino acids are an exception to the general pattern because they are not concentrated in the fetal circulation and are not transferred between maternal and fetal circulations (95, 115, 130). Anionic amino acids are taken up from either or both circulations, and in vivo millimolar concentrations of aspartate and glutamate are present within the placenta, whereas concentrations in maternal and fetal blood are in the micromolar range (108). Investigations of microvillous membrane in another laboratory (62) and of both microvillous and basal membrane in our own laboratory have demonstrated a common high affinity transporter (60, 102). This transporter is sodium-dependent, potassium-stimulated, pH sensitive, and electrogenic. Its characteristics resemble those of the X^-_{AG} system found in the apical surface of other epithelia and in nonepithelial tissues.

The observed absence of transport between mother and fetus apparently results not from the lack of uptake pathways from either circulation by the trophoblast but from the absence of an appropriate egress mechanism for release. Thus, evidence from the plasma membrane transport systems as well as physiologic studies strongly suggest that glutamate and aspartate are metabolized in the placenta rather than transported to the fetus. In vivo this metabolism may provide some benefit to the fetus, perhaps as part of an interorgan substrate cycle for fetal nitrogen metabolism or as a mechanism for protection of the developing fetus from glutamate toxicity (references cited in 60 and 102).

CATIONIC AMINO ACIDS The cationic amino acids lysine and arginine are concentrated in the fetal circulation and within the placenta. Their transport by apical microvillous membrane has not been investigated in detail, but we do know from perfusion studies that they are taken up from both surfaces into the trophoblast (152). In our laboratory two sodium-independent systems were found in basal membrane (50). One of these is the ubiquitous y^+ system, which is a relatively high-capacity, low-affinity transporter and probably serves as the major transporter of cationic amino acids across the basal membrane. The other transporter resembles the $b^{0,+}$ system, which has been described in developing mouse blastocysts (148). Both systems interact with lysine and arginine but have different specificities and widely different concentration dependence properties. This investigation provided the first known evidence of the existence of a system resembling $b^{0,+}$ outside of the developing mouse blastocyst. The very low K_m and V_{max} of this transporter suggests that in the placenta it may serve a particular function other than bulk transport

of cationic amino acids. Alternatively, in the basal membrane it may represent a residual of an earlier developmental stage of the trophoblast.

β-AMINO ACIDS The amino acid in highest concentration in placenta is the β-amino acid taurine (108). The fetus requires an exogenous supply of taurine. The active transport of taurine by the placenta yields fetal concentrations that are greater than maternal concentrations (90). Taurine is taken up by microvillous membrane by a β-amino acid carrier that interacts with other β-amino acids, but not with the neutral α-amino acids (100). This carrier requires Na^+ and Cl^- and is stimulated by gradients of these ions. The transport process is electrogenic, and the sodium-taurine stoichiometry is 2:1 or 3:1. The transporter is highly specific for β-amino acids with a K_m in the low micromolar range. A carrier with similar properties is present in various cell membranes and in the JAR placental choriocarcinoma cell line in which it is under the control of protein kinase C (90). This carrier produces a steep gradient between the placenta and maternal and fetal blood. Taurine is transferred down this concentration gradient across the basal membrane by an uncharacterized mechanism. Taurine transport with generally similar properties has been demonstrated in perfused human placentas, although there has been disagreement between two laboratories concerning selectivity (59, 76).

Monocarboxylates and Dicarboxylates

Placental metabolism of glucose generates lactate at a high rate even under well-oxygenated conditions (58, 101), and the lactate is delivered into fetal and maternal circulations. This indicates that the brush border as well as the basal membranes of the syncytiotrophoblast possess mechanisms necessary for the transfer of lactate. The lactate carrier of the brush border membrane is Na^+-independent, but it is stimulated by a transmembrane H^+ gradient (6). The operational mechanism appears to be lactate-H^+ cotransport. The system is specific for monocarboxylates such as lactate, pyruvate, and β-OH-butyrate. Dicarboxylates in general do not interact with the transporter. The transporter probably functions symmetrically, with the directionality of lactate transfer governed by the magnitude and direction of lactate and H^+ gradients across the brush border membrane. Since there is evidence that placenta generates lactic acid in vivo and delivers it into the maternal circulation, the transporter appears to function normally to eliminate lactate and H^+ from the syncytiotrophoblast. This process may play a very important role in the maintenance of intracellular pH in the syncytiotrophoblast because lactic acid, unless promptly removed, will acidify the cell. The lactate transport mechanism of the basal membrane has not yet been studied.

The brush border membrane of the placental syncytiotrophoblast also possesses a high-affinity transport system for dicarboxylates that is distinct

from the transport system serving monocarboxylates (55). The dicarboxylate transporter is energized by a transmembrane electrochemical Na^+ gradient. The substrates of the system include many intermediates of the tricarboxylic acid cycle such as succinate, malate, fumarate, α-oxoglutarate, and citrate. Pyruvate and lactate are not substrates. Because of a favorable electrochemical Na^+ gradient across the brush border membrane, the transporter may play a role in providing these metabolic intermediates that are utilized by the placenta and/or the fetus. The normal plasma concentrations of succinate, α-oxoglutarate, and citrate, for example, are in the range of 40–130 μM. Therefore, the placenta can extract these compounds from the maternal circulation via the brush border membrane dicarboxylate transporter.

Lipids and Related Compounds

Placental transport of fatty acids and triglycerides along with related metabolites including choline, inositol, ethanolamine, carnitine, cholesterol, steroid hormones, and fat-soluble vitamins has been expertly reviewed by Coleman (34). Essentially, lipoprotein lipase on the maternal surface of the syncytiotrophoblast hydrolyzes triacylglycerol carried by maternal very low density lipoprotein (VLDL). The free fatty acids are taken up by the trophoblast by unknown mechanisms that are presumably similar to those of other cells and that utilize specific membrane proteins. The trophoblast may use the fatty acids or transfer them to the fetus. The rate of fatty acid synthesis in the placenta is also quite high. LDL cholesterol is taken up by receptor-mediated endocytosis and released in lysosomes (34). Apolipoprotein E, which promotes receptor-mediated lipoprotein uptake, is synthesized and secreted by the trophoblast (111). Recently, an anion exchanger that mediates taurocholate uptake has been described in the basal membrane from human trophoblast. The transporter is electroneutral and presumably driven by bicarbonate exchange (45, 97). This system may provide an important route for fetal excretion of bile acids.

Water-Soluble Vitamins

ASCORBATE Vitamin C under physiologic conditions exists in an oxidized (dehydroascorbate) or reduced form (ascorbate). Dehydroascorbate is taken up more rapidly by syncytiotrophoblast microvillous membrane vesicles (69) and placental fragments (30) than is ascorbate. Uptake is sodium independent, and evidence has been reported both for and against mediation by the placental glucose transporter (30, 69, 70). The studies showing an effect of glucose on ascorbate transport (69, 70) used substrate concentrations one or more orders of magnitude higher than ascorbate concentrations found in maternal plasma, whereas physiologic concentrations were used in the study that failed to find a glucose interaction (30). The dehydroascorbate entering

the placental syncytiotrophoblast is metabolized to the more useful ascorbate form and released into the fetal circulation (30). The mechanism for crossing the basal membrane of the syncytiotrophoblast is unknown.

FOLATE Folate transport has been studied extensively in perfused guinea pig placenta (137, 138). Uptake occurs on both sides of the placenta by sodium-independent high affinity transporters, which can be inhibited by 5-methyltetrahydrofolate (major form in plasma) but not by methotrexate (137, 138). A putative transport protein has been identified in homogenates of human placental villi (2) and may be related to two homologous membrane-associated folate-binding proteins that have recently been cloned (110). These placental folate-binding proteins may derive from different cell or membrane locations or possible contamination by maternal tissue (110).

RIBOFLAVIN A low-capacity riboflavin transport system investigated in perfused human placenta (38–40) transfers riboflavin faster in the maternal-fetal than in the fetal-maternal direction (38, 40). This transfer was saturable and the radioactivity in the fetal perfusate was riboflavin rather than either of its two most common metabolites, flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) (38). Riboflavin was concentrated in the placenta and partially metabolized to FAD and FMN (39).

These studies demonstrate that the transfer of riboflavin across the human placenta is mediated (39) and suggest the possibility of a transporter in the microvillous membrane of the syncytiotrophoblast capable of concentrating riboflavin against a concentration gradient. Riboflavin exit into the fetal circulation may be driven by a concentration gradient between the syncytial cytoplasm and the fetal circulation.

THIAMIN Thiamin transport possesses many of the same features as placental riboflavin transport. In perfused human placentas the transfer index for thiamin was greater for transfer toward the fetus than for transfer in the reverse direction, which approximated diffusion (41). Thiamin transport was saturable at low concentrations, a transplacental gradient was established, and the tissue thiamin concentration exceeded both perfusates (41). Schenker et al (114) using the same model showed inhibition of thiamin transport by thiamin analogs. Results of these studies are consistent with the existence of a thiamin transporter in the microvillous membrane of the syncytiotrophoblast capable of uptake against a concentration gradient.

OTHER VITAMINS Concentrations of cobalamin (vitamin B_{12}), biotin, vitamin B_6, pantothenic acid, and nicotinic acid are higher in umbilical cord blood at delivery than they are in maternal blood. This finding is in agreement

with the studies of vitamins discussed previously and with the idea that water-soluble vitamins in general are transported across the placenta by mediated processes (3). In addition, human placental tissue concentrates vitamin B_{12} (105), presumably by interaction with saturable high-affinity binding sites for the transcobalamin-vitamin B_{12} complex, on the plasma membrane (49). Recently a high affinity (K_m in low μM range) Na^+-stimulated biotin transporter has been found in microvillous membrane vesicles and placental trophoblast (77).

Lipid-Soluble Vitamins

VITAMIN A Vitamin A is normally transported in the blood as a complex of retinol and retinol-binding protein (139). The mechanisms of transplacental flux of vitamin A are still not well-understood. Studies in rat (139) and monkey (144) have indicated that maternal retinol-binding protein crosses the placenta. The human placenta expresses surface receptors that bind retinol-binding protein (141), and the transplacental flux of the retinol-binding protein complex may be the main pathway for vitamin A transport across the placenta early in pregnancy. Fetal retinol-binding protein production occurs later in gestation (139). A second proposed mechanism for placental vitamin A transport is that the maternal retinol-binding protein binds to its receptor and releases retinol into the syncytiotrophoblast without the protein being internalized (144). The placenta then forms a retinyl ester that is secreted into the fetal circulation as lipoprotein. This hypothesis is supported by high concentrations of retinol found in lipoproteins in fetal circulations of monkeys (144). Similarly, a considerable fraction of retinol was esterified by human placenta and subsequently released unesterified in vitro (141).

VITAMIN D Conflicting information is available concerning placental vitamin D transport. Maternal and fetal blood concentrations of $1,25(OH)_2D_3$ and vitamin D-binding protein have been positively correlated in the rat (4). Concentrations of both $25(OH)D_3$ and $1,25(OH)_2D_3$ were found to be correlated between maternal and cord blood in humans (16). These data suggest the diffusion of vitamin D across the placenta. However, some investigators have not found a correlation between maternal and fetal blood concentrations and suggest that vitamin D is poorly transferred across the placenta (42) and instead may be produced by the placenta to meet the needs of the fetus. Unidirectional transfer of $25(OH)D_3$ and $1,25(OH)_2D_3$ has been demonstrated in a perfused placenta system (112). The clearance index of $1,25(OH)_2D_3$ was tenfold higher than that of $25(OH)D_3$ when vitamin D-binding protein was present in the perfusate.

VITAMINS E AND K Concentrations of lipid-soluble vitamins E and K are higher in maternal plasma than in umbilical cord plasma (15, 120). A study in rabbits indicates that α-tocopherol (vitamin E) does not cross the placenta in any significant amount (15). Studies in the rat (57) and human (80) have found the placenta to be a substantial barrier to vitamin K transport.

Nucleosides

Nucleosides are precursors of nucleotides, which form the building blocks of nucleic acids but additionally may be involved in other biological functions. In particular, adenosine has been shown to be a vasoconstrictor in placenta (126). Study of adenosine transport is complicated by cellular (151) as well as brush border membrane vesicle (8) metabolism of adenosine. Recently, Yudilevich (8) and co-workers were able to inhibit brush border adenosine metabolism with 2'-deoxycoformycin and thereby investigate transport. Adenosine uptake in brush border and basal membrane vesicles from human placenta was by a high-affinity mediated system that is nucleoside sensitive (8).

INORGANIC NUTRIENTS

Macro Minerals and Ions

CALCIUM Increasingly large quantities of calcium are required to support the mineralization of the growing fetal skeleton (7 mmol per day in the third trimester). Concentrations of total and ionic calcium in cord blood exceed those in maternal blood, indicating that placental transfer is an active process (109). While large quantities of calcium pass through the placenta, the syncytiotrophoblast maintains a cytosolic free calcium in the nano- to micromolar range, several orders of magnitude lower than the circulating concentrations (86). By analogy with other calcium-transporting epithelia, three types of mechanisms are likely to be used by the syncytiotrophoblast to accomplish this task: (*a*) an entry mechanism at the microvillous membrane, (*b*) a cytoplasmic mechanism for accomplishing transplacental flux while maintaining low free intracellular calcium concentrations, and (*c*) an exit mechanism to deliver calcium to the fetal circulation (20).

Recent work in our laboratory has demonstrated that mediated transport in human placental microvillous membrane possesses properties similar to those found in intestine and kidney (75). The K_m value of the higher capacity system is in the low millimolar range and suggests partial saturation at physiologic concentrations of maternal blood. The millimolar K_m, the lack of inhibition by known calcium channel blockers, and other characteristics suggest that the identified mechanism(s) are facilitated diffusion transporters rather than channels or pores. Calcium also binds to the microvillous mem-

brane by a saturable process that may protect this portion of the trophoblast from the high concentrations found in maternal blood. The relationship of calcium-binding activity to the microfilaments of the microvilli and to cytoplasmic calcium-binding proteins needs further investigation.

The processes mediating transcellular or cytosolic transfer have not been clearly defined in the placental syncytiotrophoblast. It has been suggested that vesicular packet mechanisms for transcellular or cytosolic transfer exist in other epithelia, but they have not been characterized in placental tissue (20, 146). Calcium-binding proteins have been identified in at least two laboratories. The first of these is a low molecular weight (10 kDa) protein in the rodent, similar to that in intestine. This protein is likely to be located within the endoderm of the intraplacental yolk sac, which may play a role in transplacental calcium transport in this species (26). Higher molecular weight binding proteins for calcium have been described in human placenta, and it has been suggested that they are associated with a calcium-pumping mechanism in endoplasmic reticulum or plasma membrane (143).

Several investigators have attempted to characterize ATP-dependent transport pumps or calcium-dependent ATPase activities in membranes from human placenta. Many of these earlier studies described either nonspecific divalent cation-dependent ATPases or calcium-dependent ATPases with a K_m very much greater than estimates of intracellular calcium concentrations (references reviewed in 81). We have characterized an ATP-dependent transport process in isolated basal membrane whose characteristics are similar to known ATP-dependent calcium pumps in other plasma membranes (46). We have also demonstrated ATP hydrolysis by this transporter as well as by nonspecific divalent cation-dependent ATPases activated by magnesium as well as by calcium (81). The transporter subunits react with polyclonal and monoclonal antibodies prepared against the erythrocyte calcium transporter (140 kDa) and are phosphorylated under the same conditions as the red cell transporter (81). Transporter antigenic material is located at the fetal-facing surface of the trophoblast in human placenta, the innermost (fetal-facing) layer of trophoblast in rat labyrinthine placenta, and in the endoderm of the intraplacental yolk sac in the rat placenta (14). These findings are consistent with the presence of an ATP-dependent pump situated to deliver calcium to the fetal circulation.

The studies described here provide evidence for a reasonable cellular pathway for the transfer of calcium from mother to fetus by the placental syncytiotrophoblast. At least three important questions remain: (*a*) To what extent are calcium entry and exit processes polarized between the two plasma membranes of the syncytiotrophoblast as they are in other transporting epithelia? (*b*) What is the rate-limiting step in transplacental calcium transfer? In intestinal and renal epithelia the calcium-binding protein apparently functions

to increase the rate of transfer of calcium through an area of low free calcium concentration, and this cytosolic transfer is believed to be rate limiting (20). Studies using inhibitors (135) or examining the development of mRNAs for calcium transport ATPases suggest that the ATP-dependent transport step is not rate limiting but that the binding protein-facilitated transfer may be (K. Thornburg and R. Boyd, personal communication). (*c*) What is the relative importance of transcellular and potential paracellular transfer of calcium across the placental syncytiotrophoblast layer? The concentration of calcium within the fetal circulation strongly indicates that transcellular transfer is a major process, but the relative transcellular and paracellular transfer rates are difficult to evaluate in perfusion studies with human tissue (132).

MAGNESIUM The paucity of data concerning magnesium transport—not only in the placenta but in general—is primarily due to the lack of a long half-lived radioisotope. Two recent studies using the perfused rat placenta indicate that maternal-fetal Mg^{2+} transfer is transcellular, Na^+-dependent, and independent of Ca^{2+} transport (118, 119). Perfusion with the carbonic anhydrase inhibitor acetazolamide did not affect maternal-fetal Mg^{2+} clearance, which suggests that intracellular HCO_3^- or H^+ concentrations do not alter Mg^{2+} transport (119).

PHOSPHATE Fetal serum concentrations of phosphate are higher than maternal concentrations (134), which means that the large supply of phosphate needed by the fetus in the last three months of pregnancy must be transported against a concentration gradient. Studies in whole perfused guinea pig placentas revealed a sodium-dependent transport process (133, 134) that has been localized to the microvillous membrane of the syncytiotrophoblast in human placenta (22, 94). In vesicles, sodium-stimulated phosphate uptake was concentrative and electrogenic with a pH optima of 7.0 (22) and a sodium coupling stoichiometry of 2:1 (94). Increasing temperature lowered the K_m and increased the V_{max} (94). The presence of insulin (25) or parathyroid hormone (23) reportedly decreased phosphate uptake. The parathyroid hormone effect was presumably mediated via increased cAMP (23). However, interpretation of these results is uncertain, since in both studies (23, 25) hormone concentrations were well above normal physiologic concentrations. Phosphate transport across the basal membrane could be driven by the concentration gradient between the syncytiotrophoblast and the fetal circulation.

SODIUM Transport of sodium across the brush border membrane of the placental syncytiotrophoblast occurs by at least three mechanisms: Na^+-H^+ exchange, cotransport with inorganic anions and organic solutes, and Na^+ conductance. The Na^+-H^+ exchanger catalyzes an electroneutral coupling of

Na^+ influx from the maternal circulation into the syncytiotrophoblast with H^+ efflux in the opposite direction (5, 29). The recent demonstration of the presence of a Cl^--HCO_3^- exchanger in the placental brush border membrane (56, 63) suggests the possibility of a functional coupling between it and the Na^+-H^+ exchanger. Therefore, in addition to participating in the transport of Na^+, the activity of the Na^+-H^+ exchanger is likely to contribute to Cl^- entry and HCO_3^- exit from the syncytiotrophoblast. The external substrate-binding site of the Na^+-H^+ exchanger can interact with Na^+ as well as with other cations such as Li^+, NH_4^+, and H^+. The K_m for Na^+ is approximately 10 mM, which implies that under physiologic conditions the exchanger normally operates at or near saturation. The activity of the Na^+-H^+ exchanger can be inhibited by amiloride and its analogs and also by harmaline, cimetidine, and clonidine (5, 51, 54). Chemical modification studies have indicated that histidyl, carboxyl, and thiol groups of the exchanger protein are essential for the catalytic activity (53, 93).

Two distinct types of Na^+-H^+ exchangers are known to be present in the animal cell plasma membrane and are referred to as the "housekeeping" (NHE-1) and "epithelial" (NHE-2) types. The Na^+-H^+ exchanger of the placental brush border membrane belongs to the "housekeeping" type (91). In other cells, this type has been shown to be involved in the regulation of intracellular pH and in the mediation of at least some of the biological actions of several mitogens and growth factors. These findings indicate that the Na^+-H^+ exchanger of the placental brush border membrane may participate in functions that are essential to the normal growth and development of the placenta. The recent finding that the brush border exchanger possesses an allosteric activator site for H^+ on the cytosolic side (64) suggests that the removal of H^+ from the cell by the exchanger can be finely controlled in response to changes in the intracellular concentration of H^+. The exchanger is thus an ideal system to participate in the regulation of intracellular pH.

Cotransport with other molecules also contributes significantly to the entry of Na^+ into the syncytiotrophoblast, because a number of transport systems in the placental brush border membrane utilize a transmembrane Na^+ gradient as the driving force. Various amino acid transporters (156), dicarboxylate transporter (55), serotonin transporter (7), and phosphate transporter (22) are a few examples. The third mechanism for the entry of Na^+ into the syncytiotrophoblast is a conductive pathway (29) in which Na^+ moves down its electrochemical gradient.

The exit of Na^+ from the syncytiotrophoblast into the fetal circulation occurs across the basal membrane. The primary mechanism responsible for this process is the Na^+-K^+ pump, which is localized predominantly in the basal membrane (82). Active extrusion of Na^+ via the Na^+-K^+ pump generates an inwardly directed Na^+ gradient across the brush border membrane,

which in turn facilitates the Na^+ entry mechanisms. Thus, the vectorial movement of Na^+ in the direction of maternal to fetal circulation is made possible by the differential localization of the entry and the exit mechanisms for Na^+ at the two poles of the syncytiotrophoblast.

Our most recent studies have shown that the basal membrane of the syncytiotrophoblast also contains Na^+-H^+ exchanger activity (unpublished data). Interestingly, this exchanger is pharmacologically distinguishable from the exchanger identified in the brush border membrane of this cell. The brush border membrane Na^+-H^+ exchanger belongs to the "housekeeping" type (NHE-1), whereas the basal membrane Na^+-H^+ exchanger belongs to the "epithelial" or "apical" type (NHE-2). However, the activity of the exchanger in the basal membrane is about five times less than the activity of the exchanger in the brush border membrane. The role of the basal membrane Na^+-H^+ exchanger in the physiology of the syncytiotrophoblast is not readily apparent at this time.

POTASSIUM In contrast to the transport of Na^+, very little information is available on the processes responsible for the transport of K^+ across the placenta. Apparently, the K^+-transporting mechanisms such as the K^+-Cl^- cotransport and the Na^+-K^+-Cl^- cotransport, which are known to catalyze K^+ entry across the plasma membrane of certain cell types, are not present in the placental brush border membrane (63). Potassium extrusion by the Na^+/K^+-dependent anionic amino acid transporter may occur (60, 102).

HYDROGEN ION The movement of H^+ across the placenta is important to the maintenance of acid-base balance in the fetus and the placenta. Four potential mechanisms are known. (*a*) The Na^+-H^+ exchanger of the placental brush border membrane may transport H^+ by the Na^+ gradient across the brush border membrane. (*b*) A proton pump whose presence has recently been described in placental brush border membrane (92) may also participate in the removal of H^+ from the cell. This H^+ pump belongs to the vacuolar type, and active extrusion of H^+ by the pump is energized by the hydrolysis of ATP. (*c*) A third mechanism is the coupled transport of H^+ and an organic ion such as occurs via the lactate-H^+ symport (see above). (*d*) A fourth potential mechanism for H^+ transfer is likely protein-mediated (28). This conductive pathway is voltage dependent and is allosterically activated by external monovalent anions such as I^-, Br^-, and Cl^-. Due to the presence of an inside-negative membrane potential across the syncytiotrophoblast brush border membrane in vivo, this pathway likely functions normally in the uptake of H^+.

IRON The fetus obtains its supply of iron from the maternal circulation (48). The probable first step in placental iron transfer is receptor-mediated endocy-

tosis of ferric transferrin. Transferrin receptors have been found on the surface membrane of the human placental syncytiotrophoblast (96, 117, 150). The 94-kDa molecular weight glycoprotein binds diferric transferrin with higher affinity than it binds apotransferrin (142). Studies have indicated that very little transferrin crosses the placenta (35). Most likely, iron is released in low pH endosomal compartments and the apotransferrin is recycled to the plasma membrane (43).

How iron traverses the cell and is released into the fetal circulation remains largely unknown, although the mechanism probably involves ferritin and/or other heme proteins. A transferrin receptor has been identified on the basal membrane of the syncytiotrophoblast, apparently identical to the receptor on the microvillous membrane (149). The function of this receptor is unknown, but its location suggests that it may be involved in maternal-fetal transfer of iron or perhaps that it has alternative functions unrelated to iron transport (149).

Aspects of transferrin cycling have been studied in various cell lines (145), but only recently has an appropriate model for normal trophoblast been available. Douglas & King have used this model to determine that transferrin receptors on the trophoblast surface function to bind, internalize, and recycle ^{125}I-labelled transferrin with a calculated cycle time of 11 min (43). Iron was rapidly lost from transferrin upon internalization, and most of the iron became associated with ferritin (43). Iron release from normal cultured trophoblast was gradual ($t_{1/2}$>40 h) and was associated with a low molecular weight fraction, transferrin, and ferritin (43).

CHLORIDE AND IODIDE At least three distinct mechanisms may function in the transfer of Cl^- across the placental brush border membrane. (*a*) Many investigators have reported the existence of an anion exchanger that accepts Cl^- as a substrate (56, 63, 123). The preferred exchange anion for this system appears to be HCO_3^- rather than OH^- (56), and influx of Cl^- via the exchanger is coupled to the efflux of HCO_3^-. A functional coupling between this transport system and the Na^+-H^+ exchanger is presumed to occur in vivo. Several monovalent anions compete with Cl^- for the exchange process including Br^-, I^-, NO_3^-, and SCN^- (123). The interaction of I^- with the exchanger is interesting because it may provide the necessary mechanism for active transport of this nutritionally essential element into the syncytiotrophoblast. (*b*) A conductive voltage-dependent pathway inhibited by the Cl^--channel blocker diphenylamine-2-carboxylate is known to exist (63, 123). (*c*) Chloride-coupled transport systems mediating the transport of certain organic solutes may also function in chloride entry. Examples are the taurine (77, 100) and serotonin transporters (7, 36). Both transporters exhibit an absolute requirement for NaCl, and the catalytic mechanism appears to be the cotransport of Na^+ and Cl^- with the substrate.

Micro Minerals and Ions

SULFATE/SELENATE/CHROMATE/MOLYBDATE Sulfate is an essential nutrient for optimal growth and development of the fetus, and evidence indicates that this anion is actively transported across the placenta (33). The entry into the syncytiotrophoblast across the brush border membrane as well as the exit from the cell across the basal membrane occur by anion exchange mechanisms. The brush border membrane SO_4^{2-} transporter is Na^+-independent and can catalyze uphill transport of SO_4^{2-} in response to a transmembrane HCO_3^- gradient, thus indicating the presence of a SO_4^{2-}/HCO_3^- exchange (32). Monovalent anions such as Cl^-, I^-, and SCN^- do not interfere with SO_4^{2-} transfer by this system (18). In contrast, divalent anions such as SeO_4^{2-}, CrO_4^{2-}, and MoO_4^{2-} interact significantly (19), suggesting that the essential trace elements selenium, chromium, and molybdenum may be transported to the fetus via this system. Recently, direct evidence has been obtained for the transport of SeO_4^{2-} catalyzed by the brush border membrane SO_4^{2-} transporter (121). The basal membrane SO_4^{2-} transporter also functions as an anion exchanger, but this process may not be identical with its counterpart in the brush border membrane (27).

TRACE ELEMENTS Zinc, an essential trace element, is important for growth and development. The presence of sulfhydryl groups may be important to zinc uptake by trophoblast cells (98). Transfer of zinc was increased by the presence of ligands in the perfusate of perfused guinea pig placenta (107).

Pregnant mice given aluminum orally or intraperitoneally accumulated aluminum in fetuses and placentas in concentrations 2 to 10 times those of controls (37). The doses in this study were high (both animals in the high intraperitoneal group died). Oral doses did not significantly raise maternal liver concentrations, so it is unclear whether aluminum intake would significantly raise exposure to the placenta. The heavy metals cadmium, mercury, and lead accumulate in placenta and fetal tissue, but nothing is known about their mechanisms of placental transport (122). Similarly, radioactive vanadium accumulated in fetuses of rats given intravenous injections (44). In general, the placenta does not appear to present a barrier to most trace elements, but the mechanisms of transport are unknown.

CONCLUSIONS

Substantial progress has been made in elucidating the cellular transport mechanisms of placental trophoblast. Three principal model systems have recently served as tools: placental perfusion, isolated maternal- and fetal-facing surface membranes, and, most recently, isolated trophoblast and trophoblast-derived cells. Most of the major transport systems are analagous to those of other epithelial and nonepithelial cells, and yet their distinctive

arrangement and operation in the syncytiotrophoblast is likely to be important in the functions of the placenta. These transport processes have the potential to be regulated both by intrinsic interaction with substrates and cellular nutrients and by extrinsic mechanisms involving membrane receptors and local and systemic effector substances.

The application of these and other approaches to cellular investigation will continue to develop more complete knowledge of trophoblast transport mechanisms. The integration of this knowledge with that from in vivo investigations will allow us to understand the cellular basis of trophoblast and placental function in utero and the pathways it provides for the nutrition of the fetus.

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