Transplacental Amino Acid Transfer and its Study Using Positron Emission Tomography

A short review based on a doctoral thesis

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INTRODUCTION

During its life span, the placenta fulfills its purpose of protecting and supporting the growing foetus in a variety of ways. Despite its central significance for the well-being of the foetus, our knowledge of its function remains far from adequate, albeit many studies have been conducted.
The placenta acts as an immunological barrier, though not completely, separating the maternal immunological system from the foetal transplantation antigens. The mechanisms of maternal immunological acceptance of the foetus are poorly understood, but a prerequisite for this coexistence is the maternal acceptance of the placenta as a "foetal allograft" and is achieved by the partial absence of transplantation antigens on the syncytiotrophoblast (44). The pregnancy is accepted immunologically by the mother, despite the maternal immune system retaining its principal function throughout pregnancy.

The placenta, though far less efficient, has been likened to the foetal "lung". The diffusion rate for gases per unit weight of the placenta is approximately one-fiftieth that of the lung (39). This is explained partly by the main difference between the placental exchange between two blood compartments, and the pulmonary exchange between one blood and one gaseous compartment. The respiratory gases, O_2, CO_2 and CO, are presumed to cross the placenta by simple diffusion (33). The existence of a facilitated transfer mechanism for CO has been discussed (5), but not confirmed (35).

The placenta satisfies foetal demands for energy and nutrients such as fat, sugars and amino acids by different transfer mechanisms. Foetal fat is produced from free fatty acids transferred by simple diffusion across the placental membranes and by foetal lipogenesis from maternal carbohydrates (39). The principal sugar used by the human foetus is glucose and its transfer shows several characteristics suggesting a carrier transport system responsible for transfer across the haemochorial placenta. Carrier transport systems have been demonstrated in vitro, for the D-hexoses, D-pentoses and lactate (30).

Considerable interest has been shown in the placental transfer of amino acids most of which will be transferred to the foetal circulation. Some, however, will be used as a source of energy by the placenta and for the synthesis of placental proteins. Placental protein synthesis accounts for 12-16% of amino acids transferred from the mother (6, 40). As in the case of sugars, placental transfer of amino acids is effected by transport mechanisms and not by simple diffusion (39, 65). Much

remains to be learnt about these mechanisms.

The human placenta After the human placenta has attained its definitive architecture, the maternal surface is subdivided into lobules by septa protruding into the intervillous space. These septa are folds of the basal plate, formed by a combination of trophoblast and decidual cells (41) and do not seem to play any physiological role (15). One to several foetal primary stem villi correspond to each interseptal, intervillous lobular space. Each primary stem villus breaks up just below the chorial plate into secondary stem villi, which in turn divide into tertiary villi which sweep down to attach to the decidua forming the foetal placental lobule (15, 10).

The placental villus containing the foetal capillary bed surrounded by the maternal blood is the functional unit in transplacental transfer. The blood constituents must traverse a three-layered membrane in either direction: the foetal capillary endothelium, the mesoderm of the villous core and two types of trophoblast, cytotrophoblast and syncytiotrophoblast both of which have a role in nutrition including absorbtion, phagocytosis and active transport (58). In the human, the syncytiotrophoblast and cytotrophoblast exhibit striking cytological differences. The syncytiotrophoblast has well developed endoplasmatic reticulum and golgi apparatus and numerous large mitochondria, which per unit volume appear to be more numerous than in the cytotrophoblast (20). The trophoblast represents approximately only 14% of the placental "parenchymal" volume according to morphometric measurements (4, 28). It is metabolically the most active region of the placenta (65), and its oxygen consumption is considerable, probably 25-30% of the total uterine consumption including that of the foetus,placenta and uterine muscle (38). The syncytiotrophoblast shows several features common to cells engaged in energy production and transfer. This is a prerequisite for its production of placental steroids and proteins and for its decisive role in active materno-foetal transfer.

During the second half of the pregnancy, the cytotrophoblast layer gradually disappears although residual cytotrophoblast cells persist till term. Compared with the syncytiotrophoblast cells, cytotrophoblast cells are structurally simple with limited golgi apparatus and endoplasmatic reticulum (39). The main significance of the cytotrophoblast cells seems to be as a germinal zone for the syncytiotrophoblast layer.

Transfer of amino acids. The concentrations of most amino acids in human maternal plasma are reduced during pregnancy compared to the non-pregnant state (63). The total plasma concentration of free amino acid in the foetus is about twice the maternal concentration (7). The amino acid concentration in the trophoblast is even higher compared to the maternal plasma suggesting a transfer process involving active transport from maternal plasma to the trophoblast and passive diffusion along a downhill gradient from the trophoblast into the foetal circulation (64). Christensen and Streicher (7), were among the first to suggest the existence of active transport systems for the transfer of amino acids across the placenta. Enders et al (14) determined the specificity of the principal placental transport systems for the neutral amino acids and found that

they corresponded approximately to the "A", "L" and "ASC" systems of Christensen. The "A" system is sodium-dependent and favours short amino acids with linear side chains such as alanine, glycine and the non-metabolizable model amino acid: alpha-isobutyric acid. The "L" system is sodium-independent and is most reactive with the branched chained amino acids such as valine, leucine and isoleucine and with the aromatic amino acids. The "ASC" system is also sodium-dependent and restricted in its activity to alanine, serine and cysteine (8). The specific carrier systems all overlap in their affinity for the different amino acids. Methionine is thus transported both with the "A" and "L" systems.

Control of transfer. The aim of placental transfer control of amino acids is to optimize the foetal amino acid supply to the demands. Stulc (57) questioned the necessity of a detailed regulation in view of the stable foetal environment produced by maternal homeostasis. However, the maternal homeostatic mechanisms are too often faced with extraordinary demands, such as during maternal smoking, illness and starvation (18) which may need adaptive measures of placental transfer to ensure optimal foetal supply.

Local control at the placental cellular level is influenced by the energy supply, since the active component of amino acid transfer is very energy dependent (39). Earlier results indicated that active transport across the placenta was dependent upon energy from oxidative sources (11, 53). More recent research indicates that oxidative metabolism was not required, and that the energy demands for both placental transfer and placental protein synthesis were satisfied by anaerobic glycolysis (34, 43).

The transport capacity of the placenta is adaptable in vitro, to changes in amino acid availability. Preincubation of placental tissue in amino acid free medium increased cellular concentration of the non-metabolizable amino acid alpha-isobutyric acid (52). Later, enhancement of amino acid transport under conditions of amino acid shortage has been found to be limited to transport system "A" of Christensen in both placental tissue (14) and fibroblasts (17).

In vivo studies have revealed more conflicting results. Young and Widdowson (62) reported that feeding pregnant guinea pigs on a low-protein diet caused retarded foetal growth and enhanced placental transfer and foetal uptake of alpha-isobutyric acid. Rosso (45) was unable to confirm the results of Young and Widdowson. Ahokas et al. (1) observed that a protein and energy restricted diet in rats had a variable effect on the transplacental transfer of alpha-isobutyric acid.

Hormonal regulation. The concentration of amino acids in venous blood displays a circadian rhythm, which is especially evident in the aromatic amino acids phenylalanine, tyrosine and tryptophane (60). This rhythm is not altogether explained by the fluctuations in protein intake and physical activity during day and night and thus suggests a hormonal influence on the amino acid pattern. Plasma cortisol levels seem to have an impact, though not decisive, since the rhythm has been shown to persist despite hypophysectomy (59). Both cortisone and hydrocortisone increase the uptake of alpha-isobutyric acid into the liver, which might be a reflection of the

catabolic action of steroid hormones on skeletal muscles (31).

Noradrenaline and other catecholamines increase the incorporation of tryptophane into proteins in organ cultures (61). Kidman, Weiss and Kosta (27) studied protein metabolism and amino acid accumulation in the submaxillary gland of the rat and found a higher uptake of alpha-isobutyric acid into proteins during reduced sympathetic activity of the gland. They concluded that adrenergic nerve impulses modulate the transport of amino acids from the plasma to the tissues.

Conclusive evidence for hormonal regulation of placental transport systems is still scanty. Dancis et al. (11), however, found that insulin increased amino acid uptake in human but not in guinea pig placental slices. Growth hormone, lactogenic hormone, oestradiol, oestrone sulphate and oxytocin produced no measurable response. Yudilewich and Sweiry (65) summarized the available studies of the effect of insulin on placental transfer and concluded that most investigators were unable to find any effect.

The placenta possesses a well developed cholinergic system localized mainly in the syncytiotrophoblast layer. High levels of acetylcholine (Ach) and enzymes responsible both for its production and degradation have been found in the placenta (46). Evidence for placental cholinergic receptors has been put forward by Sastry and Sadavongvivad (47). The significance of the placental cholinergic system is not fully understood, but the system might be involved in the regulatory function of amino acid transfer. Selective inhibition of choline acetyl transferase, an enzyme involved in the synthesis of Ach, lowered both the concentration of Ach and alpha-isobutyric acid uptake in the isolated human placental villous (48). Barnwell and Sastry (3) found that nicotine, a well known cholinergic blocking agent, produced a concentration dependent and reversible reduction in the uptake of all amino acids examined. They concluded that the placental cholinergic function by decreasing Ach release may affect placental amino acid uptake.

Methods for placental transfer studies

In vitro studies of placental tissue and fragments. Placental transfer studies in humans have often been based on the incubation of placental slices and tissue fragments in media containing radioactively labelled amino acids. The uptake of natural and non-metabolizable amino acids has been measured. The possibility of adding metabolic inhibitors and hormones to the incubation medium and preincubating the tissue under various conditions such as amino acid shortage have made it possible to characterize the placental amino acid transfer mechanisms. Dancis et al. (11) suggested that the transfer of amino acids to the foetus and the establishment of a maternal-foetal gradient involved a prior concentration by the placenta with a release into the foetal circulation. Smith et al. (52) noted that placental tissue took up alpha-isobutyric acid actively and that, in an hour, the concentration in the intracellular fluid reached a level five times that of the incubation medium. Preincubation of the tissue increased the rate of uptake to a concentration 25 times that in the incubation medium. Enders et al. (14) established the existence of specific transport systems for neutral amino acids in the human placenta from tissue studies using a competitive inhibition technique.

The wide variation in fluid distribution and high extracellular water content of the placental tissue reduce the precision with which the intracellular uptake of the labelled amino acid can be measured from in vitro tissue incubation studies, making it necessary to estimate the fluid spaces each time placental fragments are incubated (52).

The use of isolated cell membrane vesicles as models for transport studies seems to optimize the possibility of controlling the composition of the environment on either side of the membrane. Separation of the different membranes permits the study of properties involved in transport across the individual membrane. Results suggest that Na+ stimulates the transport of valine across the intestinal epithelial brush border membrane but has no effect on transport across the basolateral membranes (22). Dicke and Henderson (12) investigated the uptake of the non-metabolizable amino acid alpha-isobutyric acid in isolated vesicles of human syncytiotrophoblast membrane from pregnancies complicated by diabetes mellitus, hypertension or by deliveries of small-for-gestational-age (SGA) neonates. The uptake of alpha-isobutyric acid was significantly less in the SGA group.

In vitro studies of the intact placenta. Several techniques for in vitro studies of placental transfer in various species have been described. Schneider, Panigel and Dancis (49) cannulated the foetal artery and veins of newly delivered placentas and perfused the foetal side with physiological solution. The lobule supplied by the cannulated foetal vessels was identified by the colour change by the infusion medium where upon the intervillus space of the lobule was cannulated from the maternal surface for perfusion. The technique has been used in both human and animal placentae. With the in vitro perfusion technique Schneider et al. (50, 51) showed that the human placental membrane exhibited a stereospecific active transport of amino acids with a polarity in active transport directed towards the foetus and a diffusion back to the maternal circulation. The transfer rate of alanine, glycine and lysine was estimated as 2.8 μmol per min for a placenta of 450 g.

The inevitable deterioration of the preparation with risk of leakage, the use of artificial solutions in the maternal and foetal circulations and the artificial flow on both sides of the placental membrane are major drawbacks in such a model. James et al. (23), observed that the blood flow in the placenta seems to vary, in that parts of the placenta receive different amounts of blood at a given time. An in vitro model seems to be best suited for the study of qualitative aspects of placental transfer. Care should be taken in interpretating quantitative results and especially in extrapolating the observations to in vivo situations (64).

In vivo animal studies. Animal models have been employed extensively for in vivo studies of transplacental transfer . The foetus is kept either in utero or ex utero with chronic implantation of catheters after surgical exposure of maternal and foetal vessels . Using such a technique, Stegink et al. (55) showed that aspartate, like glutamate, but unlike most other amino acids, was not concentrated in the foetal circulation. With the same technique, the foetal plasma concentration of taurine was found to maintain the normal foetal to maternal concentration gradient

of 1.3-1.5 during increasing maternal plasma concentrations (56).

Ewes have been used frequently but the foeto-placental complex of the ewe shows features which differ from the human complex. In the ewe placenta (58). the maternal and foetal circulations are separated by the endothelium of maternal blood vessel and endometrial connective tissue in addition to the cellular layers separating the two circulations. This implies significant differences for the transfer of substances to the foetal lamb compared with the human foetus.

The guinea pig has been used commonly in in vivo placental transfer studies. The preparation and its merits have been described in detail by Young (64). After removal of the foetus and cannulation of the umbilical vessels, the foetal circulation is perfused with artificial fluids with the placenta in situ . An intact maternal placental circulation is thus maintained and the study of the transfer of substances is possible independent of foetal influences. The problem of the possibility of impaired maternal circulation and hence a need for pharmacological agents to maintain the maternal arterial pressure is stressed. Although the guinea pig placenta, like the human placenta, belongs histologically to the haemochorial type, dissimilarities must be considered, when relating data from transfer studies in the guinea pig to humans. The guinea pig placenta does not show any intervillous space, but has preformed channels for the circulating maternal blood. Additional differences in the placental foetal capillary network make the circulatory pattern different from the pattern in the human placenta. The importance of the rodent yolk sack in the transfer of protein molecules to the foetus compared with the vestigial human yolk sack is another difference restricting the use of guinea pigs in obtaining data relevant to human transplacental transfer.

In vivo human studies. The study of placental transfer in vivo in the human presents great difficulties due to the inaccessibility of the foetus and placenta. In vivo studies have been done after injecting a substance into the mother near the time of delivery then collecting neonatal and maternal blood samples . Christensen and Streicher (7) found from studies based on maternal amino acid loading, that high doses of methionine reduced the ratio of glycine between foetal and maternal plasma. They were the first to discuss the involvement of an active transfer mechanism of amino acids across the placenta to the foetus. Gaull et al. (16) cannulated human foetuses after hysterotomy prior to abortion. Maternal and foetal blood samples were taken simultaneously at intervals after administering an amino acid load intravenously to the mother. Materno-foetal transport of methionine, leucine and ornitine was found to take place against a concentration gradient despite a two- to three fold difference in the initial concentrations.

From the above, it is evident that our knowledge of transplacental transfer is based on in vitro studies of intact human placental lobules, placental tissue or its sub-cellular fractions. Knowledge of the conditions prevailing in vivo has been extrapolated from results of studies of different animal species performed mostly with invasive techniques. For a better understanding of the in vivo transfer in the intact human foeto-placental complex, a new approach seems necessary.

METHODS

Positron emission tomography. Positron emission tomography (PET) enables non-invasive kinetic studies of physiological processes to be performed in vivo in animal and humans. The general application of PET has been reviewed by Jones (24). Previously PET has been used mainly to investigate physiological and pathological conditions in the central nervous system (19, 32), and in the lung and heart (13)

PET utilizes positron emitting radionuclides with a short-life such as ^{15}O, ^{13}N, and^{11}C with a half life of approximately 2 min, 10 min and 20.4 min respectively. The decay of the radionuclides produces positrons, the antiparticles of electrons. A positron is immediately annihilated by an electron within a few mm from the site of decay, giving rise to two photons which escape in almost opposite directions. The annihilation radiation can be detected by external detectors. Coincidental detection in two opposing detectors provides the possibility of locating the event of the decay.

In the present series of investigations, ^{11}C was produced by bombardment of N_2 with protons at the Tandem Accelerator Laboratory, University of Uppsala. The ^{11}C atoms react with O_2 present in the target gas in minute amounts, forming $^{11}CO_2$ mainly which was utilized for chemical synthesis of [$^{11}CH_3$]-*l* - and -*d* -methionine (29).

A Scanditronix PC 384-3B positron emission tomograph (AB Scanditronix, Uppsala, Sweden) was used. It has two detector rings which surround the animal giving three adjacent cross sectional images with a thickness of approximately 11 mm and a spatial resolution of 8 mm.

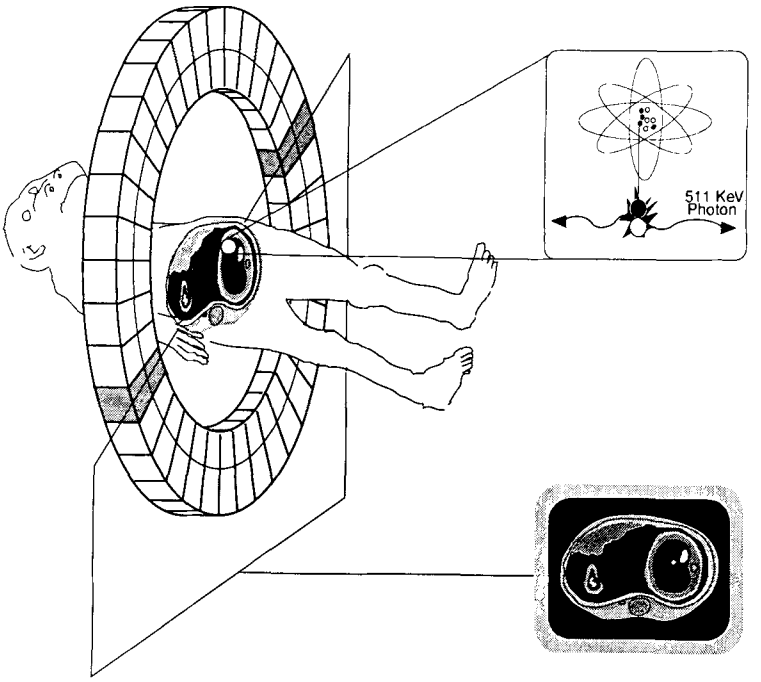

Figure 1. Schematic layout of the positron emission tomography system used in the present study.

The radioactivity was administered as an intravenous bolus injection. The distribution of radioactivity was recorded initially for 20-sec-periods, while the subsequent exposure times were progressively increased according to a predetermined program.

Cross-sectional images of the body organs based on the amount and distribution of radioactivity during each exposure were reconstructed and displayed on a screen. During the experiment, it was possible to delineate a specific region of the image and follow the amount of radioactivity in the region of interest.

Additional information was obtained by external detection of radioactivity in maternal venous blood samples collected at regular intervals and measured in a well-counter, or by continuous analysis of radioactivity in arterial blood withdrawn through a catheter in the maternal femoral artery and analyzed in a specially designed detector. In some experiments, the radioactivity in the amniotic fluid and urine was measured.

The uptake of radioactivity within the tissue was expressed as an uptake index which represents the measured radioactivity per cm^3 of tissue in relation to the amount of radioactivity administered per gram of body weight. Assuming that tissue density is approximately 1g per cm^3, an uptake index of one will represent an even distribution of the tracer in the body. The same uptake indices as for the PET measurements were calculated for the samples measured in the well-counter and in the detector for continuous blood sampling.

Positron emission tomography in the study of transplacental transfer. PET was first introduced in the study of transplacental transfer when tracer amounts of [$^{11}CH_3$]-*l* - methionine or [$^{11}CH_3$]-*d* - methionine were administered intravenously as a bolus dose to six pregnant Rhesus monkeys. The distribution in time of radioactivity was followed for up to 60 min by PET in separate regions of interest (ROI) in cross sections of the body containing the uterus with at least one placenta and the foetal liver (Fig 2). Samples of blood, urine and amniotic fluid were collected at regular intervals.Maternal plasma was separated in a high and a low molecular weight fraction by the use of gel filtration. The blood, the urine, the amniotic fluid samples and the high molecular weight fraction obtained from maternal plasma (M.W.>5000) were analyzed for radioactivity.

In order to further evaluate PET as a tool in the study of transplacental transfer, the appearance of [$^{11}CH_3$]-*l* -methionine in the amniotic cavity, which includes the amniotic fluid and foetus was measured in six Rhesus monkeys after the administration of a bolus tracer dose to the mother. Further, in four of the monkeys, the measurements were repeated after seven days on an iso-caloric protein restricted diet. Two foetuses died in utero. The transplacental transfer was again measured 14 days after return to normal diet in the two monkeys with live foetuses. Two pregnant monkeys on normal diet throughout the period underwent the same scanning protocol. Blood samples for amino acid analysis were collected from all the monkeys throughout the period of study.

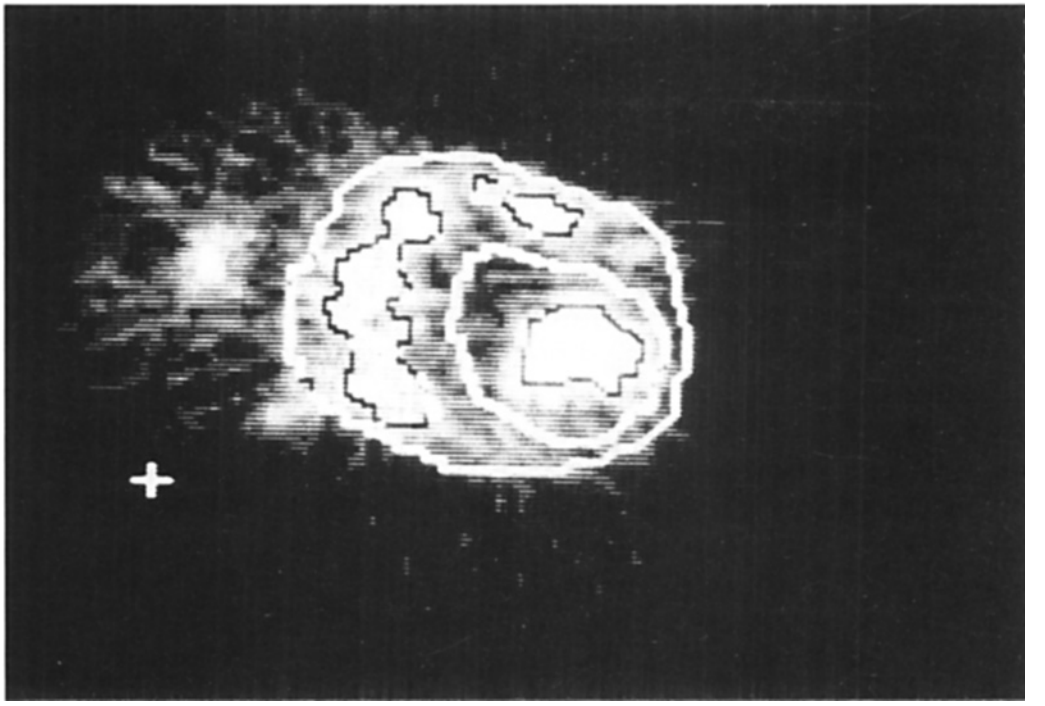

Figure 2 PET image of a cross sectional slice of a pregnant Rhesus monkey. The region of interest corresponding to the fetus is seen in the middle, surrounded by the amniotic cavity. The two placentas are seen to the left and above.

The uptake of trace amounts of [$^{11}CH_3$]-*l* -methionine in the foetus was studied with PET. After 15-20 minutes, the time required to attain a steady state in the distribution of radioactivity in the organs, the whole uterus was exposed to the detectors in sequential steps. In each step, the radioactivity was recorded for 100-200 seconds. The total amount of radioactivity within the amniotic cavity was calculated and presented as a percentage of the radioactivity given to the mother. The mean radioactivity concentration in the amniotic cavity was obtained by dividing the total amount in the amniotic cavity by its volume defined by PET.

The kinetic information obtained by PET in the investigation of the transport of [$^{11}CH_3$]-*l* -methionine from mother to foetus in the Rhesus monkey was evaluated using a simple compartment model (Fig 3).

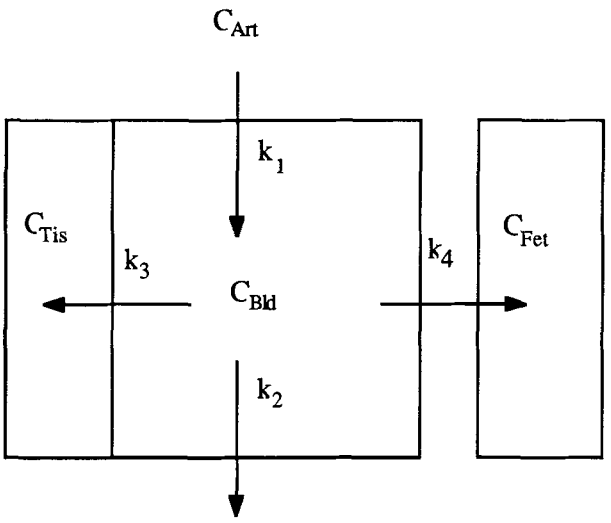

Figure 3 The compartment model used for the evaluation of kinetic data from PET studies of transplacental transfer of [$^{11}CH_3$]-*l* -methionine in the Rhesus monkey.

A catheter was placed in a femoral artery and connected to a reversible syringe pump for continuous monitoring of the radioactivity. [$^{11}CH_3$]-*l* -methionine was administered by an intravenous bolus injection. After each investigation, regions of interest (ROIs), corresponding to placental and foetal tissue were defined from the radioactivity distributions obtained by PET. The radioactivity concentrations in the placental ROI (C_{Tis}), foetal tissue ROI (C_{Fet}) and arterial blood (C_{Art}) were plotted as functions of time and fitted to an equation derived from the compartment model. Rate constants were calculated, where k_1 and k_2 describe maternal placental blood flow and k_3 and k_4, the transfer of [$^{11}CH_3$]-*l* -methionine into placental tissue and foetus respectively.

RESULTS

The PET image made it possible to identify large structures like the maternal liver, aorta, kidneys, placenta, amniotic fluid, and foetal liver. Analysis of the uptake in blood and in the regions of the PET image corresponding to the aorta and placenta revealed higher peak concentrations with slower rate of decline when [$^{11}CH_3$]-*d* - methionine was used. Uptake of radioactivity was more rapid and rose to a higher level in the foetal liver when [$^{11}CH_3$]-*l* - methionine was used. The concentration of radioactivity in the high molecular weight fraction of plasma was about the same during the first 10 - 15 min when the two enanthiomeric forms were used but increased when [$^{11}CH_3$]-*l* - methionine was used, whereas the radioactivity remained on a low level when [$^{11}CH_3$]-*d* -methionine was used. In the case of [$^{11}CH_3$]-*l* - methionine, the rate of excretion of radioactivity in urine was 0.01%/min of the given dose and 10 - 20 times higher when [$^{11}CH_3$]-*d* -

methionine was given.

The study shows that it is possible to identify and delimit the essential maternal and foetal structures in the PET image and to monitor the radioactivity necessary for the estimation of transplacental transfer of methionine. *L* - methionine is the biologically active form which is used in protein synthesis. This was reflected in the PET examinations by: (A) a more rapid clearance from the blood and the placenta, (B) a higher and more rapid uptake in the foetal liver, (C) a higher uptake in maternal high molecular weight fraction of plasma and (D) a lower urinary excretion rate when [$^{11}CH_3$]-*l* - methionine was used compared with [$^{11}CH_3$]-*d* - methionine.

The mean reduction in daily energy and protein intake on the restricted diet was 16% (range 12-22) and 54% (range 32-68) respectively. No significant changes were noted in the mean venous serum concentrations of methionine in the mothers during the period of study, although both methionine as well as a majority of the other amino acids showed a tendency towards lower values during protein restriction.

In one monkey while on restricted diet, the fraction of radioactivity in the amniotic cavity diminished compared with the fraction while on normal diet. This monkey showed a decrease in the amniotic cavity volume while on restricted diet and its foetus died in utero after the second investigation. The other three monkeys showed an increased fraction of radioactivity in the amniotic cavity during restricted diet. Two of them had an increased amniotic cavity volume and subsequently produced live births; the third displayed a constant amniotic cavity volume and the foetus died in utero after the second PET investigation.

The study of transplacental transfer kinetics showed an initial fast rise of radioactivity to a concentration of approximately 20 in the arterial blood and of approximately 3.5 in the placenta. The rise in arterial blood was followed by a rapid decrease to 0.5 in two minutes and 0.1, in ten minutes after the injection . The uptake in the placenta decreased at a slower rate and stabilized at a steady level after three to five minutes. Different investigations revealed great variations in the kinetics of the placental uptake curve in the first two minutes after the injection both in different animals and in each of the placentas of the same animal. Fitting of data from the PET investigation to equations derived from the model revealed that a four compartment model is useful in the interpretation of results from transplacental studies with PET. The rate of transfer of methionine to the foetus was estimated as 0.8-1.3 nmol/min/g placenta. The transfer of methionine to placental tissue was found to equal the transfer to the fetus. An approximate blood flow through the intervillous space of 128 ml/min was found.The correlation between placental transfer to the foetus and the maternal blood flow in the intervillous space was low.

DISCUSSION

PET has been used in neurological, psychiatric and cardiological research and has contributed to new knowledge in the fields of metabolism, blood flow and pharmacology in normal and

pathological conditions. PET will establish a position as a clinical tool in the diagnosis and treatment of intracranial tumors. Properties of PET, such as its non-invasive character and ability to provide kinetic information make it an attractive tool for perinatal research and placental physiology.

PET as a tracer technique PET offers several advantages in experimental investigations, one being that only trace amounts of the substance under study are needed. Hence pharmacological effects of the labelled compound will not occur. In most of our studies, the amount of radioactivity that was administered varied in the range of 50 - 200 MBq. The specific radioactivity obtained at the end of synthesis of the D/L-methionine was in the order of 370 MBq per μmole. The levels of free amino acids in serum during pregnancy and foetal life in the Rhesus monkey have been investigated by Kerr (25). He found concentrations between 32 and 42 μmole/l between 100 and 150 days of pregnancy. These results are in accordance with our own estimations ranging between 10 and 40 μmole/l. The amount of methionine administered during PET investigations was in most cases well below 0.5 μmole. The labelled methionine could therefore be regarded as a tracer for the pool of methionine in the Rhesus monkey.

Incorporation of the tracer It was shown that [$^{11}CH_3$]-*l* - methionine was incorporated in the high molecular weight fraction of plasma to a much greater degree than [$^{11}CH_3$]-*d* - methionine. The difference was visible 20 minutes after the administration of the different tracers. This is in agreement with the concept that the *l* -forms of the amino acids are the main precursors in protein synthesis. At the same time this fact limits the time during which kinetic information of transplacental transfer of *l* -methionine can be obtained to 10-15 minutes. After this time an increasing amount of radioactivity seems to be incorporated into peptides and proteins. Routes of degradation yielding an increasing amount of radioactivity in the low molecular fractions of plasma even shortly after the administration of radioactivity have also been shown (37). Knowledge of the metabolic pathways involved and additional analytical techniques are necessary for a proper interpretation of PET data derived from the metabolized radio labelled molecule. It may seem contradictory that transplacental transfer in one part of the investigation was studied after a steady state had been established in the organs. However, at that point transfer was studied from a static point of view, giving the relative amount of the tracer administered to the mother that was retrieved in the foetus. Although some of the radioactivity at the time of the investigation was incorporated into proteins or metabolites, we assumed that the radioactivity reached the foetus bound to methionine and that the measured radioactivity, irrespective of where it was incorporated or not, should be an indicator of the accumulation of methionine in the foetus.

It was also assumed that the transfer of methionine was an one-way transfer during the time of the investigation and that the amount of tracer transferred to the foetus was incorporated and trapped in foetal proteins. The assumption was based upon results which showed that the uptake of [$^{11}CH_3$]-*l* -methionine in the high molecular weight fraction of plasma was not seen until 15 min

after administration of radioactivity - a period preceded by cellular extraction of the tracer from the blood to prepare for the protein production in the cells.The assumption was further supported by the result from one PET experiment, in which [$^{11}CH_3$]-*l* -methionine was administered to the foetus. No radioactivity could be seen in the placenta or in the maternal tissues during the first 15 min. (unpublished observations).

Practical difficulties complicated the analysis of the PET data. Maternal and/or foetal movements were indicated by unexpected irregularities in the uptake curve usually seen simultaneously in all three PET slices. The initially defined ROI did not any longer cover the intended structure. This problem was solved by either ending the analysis of data when movements first were discerned, provided the movements took place during later parts of the investigation, or by defining a new ROI to be used for the analysis of the remaining part of the experiment.

The analysis of ROIs covering the placenta posed specific problems. It was not always possible to decide whether lack of congruence between the placenta and its ROI was merely the effect of maternal movements. In all other ROIs except that of the placenta there was a good correspondence between the specific tissue and the corresponding ROI. This shift between the placenta and its ROI, which would normally be looked upon as the effect of maternal movements, might as well be caused by changes in maternal placental blood flow or -volume, possibly due to uterine contractions. Previous studies have given both morphological and functional support to the concept of non-uniform blood flow through the intervillous space of the placenta. (23, 2).

The delineation of the amniotic cavity was facilitated by the difference in radioactivity between the amniotic fluid and uterine wall surrounding the placenta(s) The delimitation of the caudal and cranial parts of the amniotic cavity was obscured by the high uptake in the adjacent maternal bowel and liver and the relative scarcity of amniotic fluid in the rostral and caudal regions. This problem was most evident in the study involving restricted food intake (paper II). The intention not to incorporate any irrelevant tissue into the amniotic cavity ROI, might lead to underestimation of the volume of the amniotic cavity .

Close monitoring of maternal and foetal movements during the PET experiment together with complete delineation of the placenta by other means than PET, such as x-ray computed transmission tomography and/or ultra sonography should help to elucidate these problems.

Transplacental transfer. Two different approaches to the use of PET in transplacental transfer were developed; one which describes PET in a quantitative situation and the other, where PET is applied to the kinetic situation of transplacental transfer. Both methods are hampered by factors such as lack of adequate resolution in the PET and the risk of foetal movements but both possess individual advantages. The measurement of the relative amount transferred to the foetus offers several advantages. The calculations are less complicated and yield results that are easier to interpret, although they reflect only a static aspect of transplacental transfer. It is only necessary to monitor the radioactivity from one source, the foetus, which excludes the use of intraarterial

catheters for continuous monitoring of radioactivity in maternal arterial blood, necessary in compartmental analysis.

The limited resolution prevents PET measurement of foetal volume, which would be the most accurate volume to measure. The amniotic cavity volume could be measured by a dilution technique and the foetal volume could then have been subtracted. Since the intention was to make serial measurements, amniotic puncture was avoided so as not to induce premature delivery.

Only minute amounts of radioactivity were found in the amniotic fluid compared with the foetus. The uptake in the amniotic cavity, which was identified in the PET image by the contrast between the low uptake in amniotic fluid and the high uptake in the adjacent tissues, was therefore considered to be approximately equal to that of the foetus. The minute amount of radioactivity measured in the amniotic fluid also poses a problem, in that changes in the volume of amniotic fluid alone will influence the tracer concentrations. Consequently, tracer concentration in the amniotic cavity is a less sensitive measure of transplacental transfer. Measurement of total radioactivity in the amniotic cavity would probably relate more closely to transplacental transfer, but measurement of total radioactivity is adversely affected by incomplete delineation of the amniotic cavity in its caudal and cranial poles as discussed above.

A compartment model of the placenta and the fitting of experimental data to the equation derived from the model have several advantages. The dynamic events inherent in the transfer process could be described in terms of rate constants which can be used for further description of transfer. Results from several investigations can be compared and treated by standard statistical methods.

Kinetic analysis of transfer with PET necessitates continuous monitoring of radioactivity concentration of maternal arterial blood, which could only be achieved by means of external detection in blood withdrawn continuously through a catheter. Percutaneous arterial catheterization has proved difficult to accomplish due to the small diameter of the suitable femoral vessels. Surgical exposure of the vessels was refrained from, since this would make repeated puncture of the vessels difficult due to scarring, violating the non-invasive character of the method. The small diameter of the vessels necessitated also the use of very thin catheters which inevitably increased the risk of clotting.

Apart from difficulties in maintaining the catheter patent during the experiment (see above), the resistance to flow in the catheter will introduce divergences in the sampled radioactivity curve. Such errors can be reduced by minimizing the length of the catheter by placing the detector as close as possible to the animal, and by withdrawing blood through the catheter at utmost speed. In practice, the small diameter of the femoral artery sets the limit to the size of the catheter. The external detector which is used today demands an effective length of catheter of approximately 30 cm. A smaller design of the detector would allow closer positioning of the detector to the animal and thus a reduction of the effective length of the catheter. The speed at which blood was withdrawn during the experiments was set at 1 ml per min, which resulted in a blood loss of 20 ml per investigation. The blood loss had to be kept at a minimum, so as not to disturb the physiology

of the animal.

At the moment, it is not possible to monitor adequately the maternal blood concentration in the Rhesus monkey by means of PET. The small cross sectional area of the maternal aorta will give poor counting statistics and will be sensitive to maternal movements. The problem will be solved with a whole-body PET camera. The whole animal can then be examined simultaneously and the uptake from the maternal heart will provide the input function for the radioactivity in maternal arterial blood.

A low correlation was found between maternal placental blood flow and transplacental transfer of methionine in the evaluation of the data derived from the compartment model. This observation is in agreement with Clapp's (9) statement, that during normal conditions, flow rates through both the uterine and foetal umbilical circulations are relatively unimportant variables in maintaining effective transplacental exchange.These results may be of importance in the evaluation of ultrasonographic monitoring of the umbilical blood flow, introduced to detect intrauterine foetal growth retardation in human pregnancies.

Young (62) found an increased rate of transfer of amino acids to the foetus during protein restriction, as did Ahokas et al. (1). It is evident that neither from the part of the investigation dealing with static transfer, nor in the part describing the compartment model, could it be shown that protein restriction had any specific effect on transplacental transfer, even if a tendency to increased concentration of radioactivity could be shown in the amniotic cavity during protein restriction. The two monkeys that were fed on a protein restricted diet prior to the investigation displayed transplacental transfer rate constants that did not deviate significantly from those observed in the group fed on normal diet. However, the results indicate a mean transfer rate to the foetus of 1.1 nmol/min/g placenta, which is lower than the value of 7.6 nmol/min/g placenta reported by Schneider et al. (49) using an in vitro model with an isolated human placental cotyledon. Our results indicate a transfer capacity of 38 mg methionine/24h for an ordinary Rhesus placenta weighing 165 gram, which is the mean weight of a placenta during the last month of pregnancy (53). Holt (21) calculated the amount of amino acid required to maintain adequate growth in infants to be between 33-45 mg methionine/kg/day. Although our study and that of Holt are not strictly comparable, our estimate of the transplacental transfer rate seems to be similar to that of Holt and the amount of amino acids transferred adequate to meet the physiological needs for foetal growth.

It was found that the transfer of methionine into the placental tissue equalled that to the fetus. The placenta will use amino acids for incorporation into structural proteins for its own growth and for the production of peptide hormones. It has also been found that the placental metabolic rate is high during in vivo conditions and that the placental consumption of oxygen accounts for half of that of the pregnant uterus and its contents (38, 42)

Future prospects. The main purpose of the present series of investigations was to develope PET as a method for studies of the foeto-placental complex. It should be possible to extrapolate the

knowledge acquired about the kinetics of placental transfer in the Rhesus monkey to human pregnancy. The studies were conducted also as a preparation for the application of the PET technique to studies of human pregnancy.

Before the PET technique can be applied in the study of human pregnancy, two additional prerequisites must be fulfilled. (A) It should be proven beyond doubt that the technique does not carry any radiation hazard to the mother and her child. (B) A PET camera with a detector opening large enough to contain a pregnant woman must be available.

In the case of radiation hazards to the mother and foetus, several issues need further elucidation. The decay of positron emitting radionuclides will yield radiation derived from the positrons during their short penetration into the tissue (ß-radiation) and from the subsequent annihilation photons . The consequences of tissue interaction with the photons will resemble the consequences of tissue interaction with the ß-particles, although the absorbtion of photons does not have the same finite range, since photon interaction will give rise to harmful high energetic electrons all along the path of the photons (54).

In investigations involving positron emitting radionuclides the absorbed dose cannot be calculated generally, since the dose is dependent on the half life and ß-energy of the radionuclide and on the distribution and excretion of the labelled ligand in the body. Thus the calculation of the effects of radiation must be made separately for each series of investigations.

The radiation dose to the foetus from a PET investigation involving the administration of 100 MBq of a ^{11}C-labelled ligand to the mother can be estimated roughly. (A) If the administered ligand is kept solely within the maternal circulation and is not transferred to the foetus, the foetal dose will be exclusively derived from the maternal photon radiation and amounting to about 0.2mSv.

(B) Ligands which cross the placenta and become evenly distributed in the organs of the mother and foetus would increase the ß-dose from the positron radiation, while the photon dose would remain evenly distributed. The total radiation dose would amount to 0.4 mSv. (C) Ligands that cross the placenta and concentrate in the foetus to twice or five times that of an even distribution would yield a foetal radiation dose of 0.6 and 1.2 mSv respectively. These figures can be compared to the normal background radiation which, excluding the contribution from radon, is about 1.0 mSv/year in Sweden and the mean absorbed dose from a radiographic pelvimetry for foeto-pelvic disproportion which is 0.7-1.9 mSv (36). A new law on radiation protection will recommend a maximum dose of 5 mSv to the foetus during pregnancy.

It seems that application of PET in the investigations of human pregnancies in a near future will be both technically possible and ethically justifiable with regard to the radiation hazards involved. Our efforts so far have been concentrated on exploring the basic questions concerning the application of the PET technique as a tool for the study of placental physiology. Additional work will be needed in order to develop a method for placental blood flow estimations. Progress has so far been hampered by the difficulty of establishing and maintaining patency of the maternal arterial catheter necessary for the continuous monitoring of arterial blood radioactivity. Arterial

catheterization will be greatly facilitated when applied to the human in view of the large size of the arteries in humans compared to the Rhesus monkey and may even prove superfluous in view of the possibility in the future of monitoring the maternal arterial radioactivity concentration directly by PET.

Foetal growth is dependent on energy and nutrient supply. Foetal supply involves functions such as foetal blood flow, placental transfer capacity and maternal placental blood flow. With research tools suited for the estimation of placental transfer and maternal placental blood flow, it will be possible to investigate the basic mechanisms relevant for foetal growth, not only in human pregnancies that are compromised by different causes and result in retarded growth, but also in conditions associated with accelerated foetal growth, as maternal diabetes. Both carbohydrates, fat and several other amino acids besides methionine relevant for foetal growth can be labelled with ^{11}C and used in PET studies.

The equipment employed in the present studies does not allow investigation of small foetal structures. The foetal liver was chosen because it is a homogeneous organ of sufficient size and of great importance for protein synthesis. More modern PET cameras have better resolution, permitting the examination of smaller structures. The PET technique can be used in a wide range of investigations during pregnancy including foetal developmental pharmacology, foetal brain research which might give clues to the etiology of common diseases such as cerebral palsy and in investigations of foetal influence of maternal drug abuse.

A new PET center in Uppsala is being built and will be ready in 1991. This center will be run jointly by the University of Uppsala and the hospital, and will house facilities for nuclide production and analytical chemistry as well as a new whole body and brain camera. The advent of a new PET center will hopefully facilitate retrieval, display and computer manipulation of acquired data from the PET investigations.

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