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Method for the Study of Metabolite Transfer from Rat Mother to Fetus

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Abstract. Infusion medium containing a tracer was introduced through the left uterine artery to 20.5-day pregnant rats. In this way, the left uterine horn received the tracer directly while it reached the right horn after dilution in the mother's circulation. When U-^{14}C-*D*-glucose or U-^{14}C-*L*-alanine was infused, radioactivity in fetal blood from the left uterine horn was 3 or 8 times higher respectively than in the right horn and 2 or 6 times higher than in maternal blood. When the infusion was carried out with ^{125}I-labelled growth hormone, which is known not to cross the placental barrier, very little radioactivity was found in fetuses compared with that in the mother's plasma and no differences were observed in blood radioactivity levels of fetuses from either horn. After infusion of U-^{14}C-*D*-glucose through the maternal jugular vein instead of the left uterine artery, radioactivity was the same in fetuses from the left and right horns, indicating that manipulations of the left uterine side did not greatly affect its circulation. These findings, together with the determination of specific radioactivity of the tracer used in both maternal and fetal plasma, validate the technique as a semiquantitative means to determine the direct transfer of any product through the placental barrier in the rat.

Studies of the in vivo passage of substances from maternal blood into fetal circulation have been performed in sheep [1, 15] and rabbits [5]. The mechanism of this transfer has been investigated using in situ placental perfusion [12, 13, 16], isolated placenta [19, 25], or in vitro incubation systems [20] in humans, sheep, or guinea pigs. The rat has been used extensively as an experimental model for studies of intermediary metabolism in pregnancy, but placental transfer in this species has been examined indirectly, for example by giving a radioactive substrate to the mother and determining its appearance in the fetus. Due to the difficulties in interpreting results from this type of experiment, the present study was performed to test an easy method for determining placental transfer in situ in the rat. The technique consists of infusion of a radioactive metabolite through the left uterine artery. In this way the placentas on the left side receive the infusion medium

directly before it is diluted in the maternal circulation. Comparison of fetal radioactivity present in the left and right uterine horns may permit determination of whether and how the metabolite crosses the placenta before it is transformed by the mother.

Materials and Methods

Fed pregnant Wistar rats at day 20.5 of gestation (286 ± 6 g) were anesthetized with sodium pentobarbital intravenously (33 mg/kg body weight). After laparatomy, the arteries corresponding to the hypogastric trunk, superior gluteal, superior external pudendal, and the deep circumflex [7] of the left side were clamped. A cannula (PE-10; Intramedic, USA) was introduced countercurrent into the left external iliac artery, distal to the level of the external superior pudendal artery. The cannula was introduced just to the beginning of the superior vesical artery (from which the uterine artery derives). The superior vesical artery was clamped distal to the left uterine artery exit level. With this procedure, the perfusion medium introduced through the cannula combines with the blood circulating through the left uterine artery. After surgery, the conceptus was conveniently located in the maternal abdomen which was closed for the rest of the experiment.

In some experiments, the mother rat was also infused through its general circulation by cannulating the right jugular vein (PE-50; Intramedic). The entire surgical procedure lasted around 45 min. Infusion of tracer was always done in 0.9% saline media using a Gilson peristaltic pump at a rate of 300 µl for 15 min (20 µl/min), except in experiments using U-^{14}C-alanine when 250 µl were perfused for 20 min (12.5 µl/min).

After collection of maternal blood from the aorta into heparinized syringes, either the fetuses or placentas from the left and right uterine horns were immediately excised. When using U-^{14}C-*D*-glucose as tracer, fetuses were decapitated and blood was collected into heparinized receptacles. All fetal blood from each uterine side was pooled separately. Aliquots of whole blood were placed into scintillation vials and after being decolored with H_2O_2, they were counted for total radioactivity in a xylene/Triton-100/PPO/POPOP based scintillation liquid. The remaining blood was used for plasma separation and aliquots were used for counting total radioactivity as indicated for whole blood and deproteinization [22]. Glucose was measured [10] in aliquots of the protein-free supernatants. Aliquots of 400 µl from these supernatants were passed over microcolumns of Duolite A-561, generously provided by Diamond Shamvack Co., Redwood City, Calif., and Dowex 1 × 2–400 (Sigma Chemical Co., St. Louis, Mo.) prepared as previously described [8] except that Duolite resin was placed in both the top and bottom of the column whereas Dowex was in the middle. Columns were rinsed with 3 ml of deionized distilled water and eluates were lyophilized and counted. Recovery of U-^{14}C-*D*-glucose added to blood before precipitation was always above 99% with this technique, whereas the recoveries of added U-^{14}C-*L*-alanine, U-^{14}C-*L*-lactate and U-^{14}C-pyruvate were always less than 0.6%. Placentas and bled fetuses from each uterine horn were pooled and placed in a mortar with liquid N_2 to be powdered. Aliquots of the powdered placenta or fetuses were digested in boiling 30% KOH for 30 min and after neutralization with HCl they were counted for radioactivity.

When U-^{14}C-*L*-alanine was used as tracer, sampling was similar to that followed for ^{14}C-glucose except that a whole fetus from each of the ovarian extremes, instead of the pool of exsanguinated fetuses, was used for total radioactivity measurement. Plasma aliquots were deproteinized with 10% $HClO_4$ and neutralized with saturated $KHCO_3$. Protein-free supernatants were used for glucose [10] and alanine [24] estimations. Labelled alanine was isolated [6] from 100 µl of the deproteinized supernatants by passing them over microcolumns containing cationic (AG50 W-X8, 200–400 mesh, in hydrogen form; Bio Rad Laboratories, Richmond, Calif.) and anionic resins (AG 1-X8, 200–400 mesh, in chloride form; Bio Rad Laboratories). Columns were rinsed sequentially with 5 ml deionized distilled water, 5 ml of 0.5 *M* formate and 5 ml of HO-NH_4. Recoveries in the last eluate for U-^{14}C-*L*-alanine added to blood before precipitation were 100.7 ± 2.1% whereas the recoveries of added U-^{14}C-*D*-glucose and U-^{14}C-*L*-lactate were 0.4 ± 0.3‰ and 0.7 ± 0.5%, respectively.

In experiments in which ^{125}I-labelled growth hormone (^{125}I-GH) was infused, aliquots of blood from the mother and each of the fetuses were used for counting total radioactivity in a gamma solid-phase scintillation counter (Nuclear Chicago, model 1185). After counting blood samples, proteins were precipitated with 10 vol of 7% trichloroacetic acid and

radioactivity was measured again. Radioactivity in the placenta from each uterine horn was measured directly.

Results of radioactivity are presented either as dpm (when using ^{14}C radiochemicals) or as cpm (when using ^{125}I) always corrected by considering 1×10^6 dpm (or cpm) as the total infused radioactivity per rat. Statistical comparison between groups was done by using the paired t test [21].

Radiochemicals [U-^{14}C-*D*-glucose (199.6 mCi/mmol) or U-^{14}C-*L*-alanine (10.0 mCi/mmol)] were obtained from the Radiochemical Centre, Amersham, England. They were lyophilized and diluted with 0.9% NaCl to obtain final solutions of 20 μCi/ml that were used directly as infusion medium. Rat GH was obtained from NIAMDD (Bethesda, Md.), labelled with ^{125}I-NaI (Radiochemical Centre) and purified [11], giving a final solution of 50 μCi/ml in saline, that was used for infusion.

Results

Infusion with U-^{14}C-D-Glucose through the Left Uterine Artery

After 15 min of continuous infusion with U-^{14}C-*D*-glucose through the left uterine artery in pregnant rats on day 20.5 of gestation, total radioactivity in fetal blood coming from the left uterine horn was 3 times higher than in blood from the right side (table I) and it was almost twice as high as in maternal blood. Similar differences were found in the total radioactivity in placenta and in fetuses from the left versus the right uterine horn. Plasma ^{14}C-glucose/ml was higher in fetuses from the left than in those from the right uterine horn but lower than in the mother's plasma (table I). The percentage of plasma ^{14}C-glucose radioactivity, compared with total plasma radioactivity, was greater in mothers than in fetuses with no differences between either fetal uterine side (table I). Plasma glucose concentration was slightly but not significantly higher in mothers than in fetuses (table I). Plasma glucose specific activity was higher in mothers than in fetuses being also higher in fetuses from the left than the right uterine horn (table I) although the differences were not statistically significant except between fetuses from the right uterine horn and the mothers.

Table I. Infusion through the left uterine artery with U-^{14}C-glucose in the 20.5-day pregnant rat (mean ± SEM of 4–6 animals)

	Mother	Fetuses		
		left uterine horn	p	right uterine horn
Total radioactivity in blood, dpm/ml	5,515 ± 490	9,798 ± 1,253**	< 0.001	2,707 ± 334**
Placenta, dpm/g		10,159 ± 1,389	< 0.001	3,240 ± 363
Fetuses, dpm/g		10,636 ± 1,732	< 0.01	3,017 ± 478
^{14}C-glucose in plasma, dpm/ml	3,823 ± 981	1,382 ± 283*	< 0.05	752 ± 112*
% ^{14}C-glucose vs. total ^{14}C in plasma	65.5 ± 3.9	25.9 ± 7.7**	NS	25.6 ± 3.1***
Glucose in plasma, m*M*	4.92 ± 0.82	3.83 ± 0.43	NS	3.65 ± 0.31
Glucose-specific activity, dpm/μmol	748 ± 201	376 ± 76	NS	213 ± 40*

The statistical significance between values from the left and the right uterine horns is shown by p and that of each group versus the mother by asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; NS = not significant ($p > 0.05$).

Infusion with U-^{14}C-L-Alanine through the Left Uterine Artery

Following the same experimental protocol used for U-^{14}C-*D*-glucose, the left uterine artery was infused with U-^{14}C-*L*-alanine. As shown in table II, total radioactivity in fetal blood from the left horn was 8 times higher than in mothers. Fetal blood radioactivity in the right uterine horn was significantly lower than in the mother (table II). ^{14}C-alanine present in plasma was higher in fetuses in the left uterine horn than in the right horn or the mother, and the percentage of radioactivity in plasma as ^{14}C-alanine, compared with total plasma radioactivity, was significantly higher in the left than in the right side fetuses, without significant differences compared with values in the mothers (table II). Plasma alanine concentration was similar in fetuses from the left and right sides and in both cases, values were much higher than in maternal plasma. Plasma alanine specific activity was significantly higher in the fetuses from the left horn than in the mothers and in those fetuses from the right horn (table II). These specific activity values of alanine were higher than those of glucose (table I) and the difference was specially manifest in the plasma of both the mothers and the fetuses from the left horn.

Infusion with U-^{14}C-D-Glucose through the Jugular Vein in the Mother

To validate the method, it was necessary to determine whether the surgical procedure and infusion through the left uterine artery altered blood circulation and consequently the availability of metabolites to the fetuses in the left side compared with the right. For this purpose, the left uterine artery was infused with saline while U-^{14}C-*D*-glucose was infused through the maternal jugular vein. As shown in table III, radioactivity in blood, ^{14}C-glucose in plasma, and the percentage of ^{14}C-glucose in plasma as compared with total plasma radioactivity were significantly higher in mothers than in fetuses and none of these parameters differed in fetuses from the

Table II. Infusion through the left uterine artery with U-^{14}C-alanine in the 20.5-day pregnant rat (mean ± SEM of 4–6 animals)

	Mother	Fetuses		
		left uterine horn	p	right uterine horn
Total radioactivity in blood, dpm/ml	2,045 ± 140	12,136 ± 804***	< 0.001	1,395 ± 66**
Placenta, dpm/g		15,206 ± 2,619	< 0.001	2,049 ± 107
Fetuses, dpm/g		7,926 ± 1,888	< 0.01	1,604 ± 92
^{14}C-alanine in plasma, dpm/ml	839 ± 64	5,281 ± 495***	< 0.001	430 ± 22**
% ^{14}C-alanine vs. total ^{14}C in plasma	32.4 ± 3.6	39.7 ± 3.7	< 0.01	25.8 ± 1.5
Alanine in plasma, μ*M*	408 ± 43	1,526 ± 177***	NS	1,174 ± 101***
Alanine-specific activity, dpm/μmol	2,056 ± 157	3,461 ± 318**	< 0.001	366 ± 16***

The statistical significance between values from the left and the right uterine horns is shown by p and that of each group versus the mother by asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; NS = not significant ($p > 0.05$).

left and right sides. Plasma glucose concentration was again slightly higher in mothers than in fetuses, with no difference in this parameter between samples from either horn. Specific activities of plasma glucose were higher in mothers than in fetuses from either the left or right horn sides between which there were no differences (table III).

Infusion with ^{125}I-GH through the Left Uterine Artery

To further validate the method, the left uterine artery of a pregnant rat was infused with ^{125}I-GH, a hormone known not to cross the placenta [2]. As shown in table IV, radioactivity in fetal plasma was very low compared with the maternal value and did not

Table III. Infusion through the right jugular vein with U-^{14}C-glucose and through the left uterine artery with saline in the 20.5-day pregnant rat (mean ± SEM of 5 animals)

	Mother	Fetuses		
		left uterine horn	p	right uterine horn
Total radioactivity in blood, dpm/ml	6,404 ± 395	5,020 ± 327**	NS	5,361 ± 473**
Placenta, dpm/g		5,847 ± 191	NS	5,842 ± 395
Fetuses, dpm/g		5,294 ± 290	NS	5,417 ± 300
^{14}C-glucose in plasma, dpm/ml	5,131 ± 302	2,290 ± 293***	NS	2,222 ± 385***
% ^{14}C-glucose vs. total radioactivity in plasma	64.5 ± 1.3	38.8 ± 4.4***	NS	33.7 ± 3.8***
Glucose in plasma, m*M*	5.39 ± 0.31	4.04 ± 0.55	NS	3.64 ± 0.46*
Glucose-specific activity, dpm/μmol	873 ± 46	557 ± 68**	NS	580 ± 43**

The statistical significance between values from the left and the right uterine horns is shown by p and that of each group versus the mother by asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; NS = not significant ($p > 0.05$).

Table IV. Infusion through the left uterine artery with ^{125}I-GH in a 20.5-day pregnant rat

	Mother	Fetuses (mean ± SEM)		
		left uterine horn	p	right uterine horn
Total radioactivity in plasma, dpm/ml	53,538	167 ± 14	NS	158 ± 15
Plasma trichloroacetic acid precipitable fraction, dpm/ml	46,787	67 ± 12	NS	39 ± 5
Placenta, dpm/g		4,122 ± 198	NS	3,891 ± 182

p corresponds to the statistical significance between the values from the left and the right uterine horn: NS = not significant ($p > 0.05$).

differ between left and right uterine values. While a minor proportion of plasma radioactivity in fetuses was precipitable with trichloroacetic acid, most of it (85%) was precipitable in maternal plasma. Radioactivity in placentas did not differ in the left and right uterine sides (table IV).

Discussion

By placing a cannula at the beginning of the left uterine artery and clamping all other arterial derivations around that area, it was possible to infuse in the late pregnant rat a radioactive metabolite directly through the circulation irrigating the left uterine horn. In this way, passage of radioactivity to the right uterine horn occurred after dilution in the maternal systemic circulation. This experimental design permitted maximal specific activity of any infused tracer in the left uterine artery so that any compound crossing the placenta would produce higher radioactivity in fetal blood from the left than the right uterine horn. This occurred when infusions were done with either U-^{14}C-*D*-glucose or U-^{14}C-*L*-alanine although the fetal-maternal differences were much greater with the latter than the former tracer. These results are in agreement with the known fact that *D*-glucose crosses the placenta by facilitated diffusion [17, 23] whereas *L*-alanine does it by active transport [3, 4].

When U-^{14}C-*D*-glucose was infused through the left uterine artery, the percentage of ^{14}C-glucose in the plasma, compared with that in total plasma, was much lower in the fetus from the two horns than in the mother. Although the actual rate of glucose utilization was not measured, these findings indicate that glucose metabolization is faster in the rat fetus than in the mother, in agreement with the conclusion obtained from studies in sheep [9]. When infusion was done with U-^{14}C-*L*-alanine, the amount of label found as alanine in the plasma of fetuses from the horn of the same infusion site was much greater than that found as glucose when the infusion was done with U-^{14}C-*D*-glucose. This difference may be interpreted as a consequence of the faster conversion of glucose into other metabolites. In addition, plasma alanine concentrations in both mother and fetuses were approximately 12 times lower than those of glucose, resulting in a specific activity of alanine much greater than that of glucose. This was specially true in the plasma of the fetuses from the left uterine horn, indicating that the actual transfer of alanine from maternal to fetal circulation in the rat is lower than that of glucose, which also agrees with observations carried out in the sheep [14].

After clamping the arterial derivations of the left iliac artery and canalizing the circulating flux through the left uterine artery, the circulatory rate might be increased in that artery. This possibility was tested by infusing U-^{14}C-*D*-glucose through the maternal jugular vein and saline through the left uterine artery. No differences appeared in the passage of the tracer to fetuses in the left and right uterine horns, demonstrating either that no changes occurred in the circulatory rate at the left uterine artery by its infusion with saline, or that any change produced did not affect transfer of the tracer from maternal circulation to the fetuses. The volume of the infusate used (250–300 µl in 15–20 min) is almost negligible compared with the circulating flux which is approximately 2 ml/min according to our data, obtained by exsanguination, and to other reports [2, 18]. Thus, the

tiny volume infused does not alter the circulatory flux or dilute the blood circulating through the left uterine horn.

Our results with ^{125}I-GH further validate the technique used, as this hormone is known not to cross the placenta [2] due to its high molecular weight. Following infusion of the left uterine artery with this hormone, almost all radioactivity remained in maternal circulation and only a very small amount appeared in fetal circulation, most of it corresponding to nonprotein material with no differences in the amount of label appearing in the left and right fetal uterine horns.

In conclusion, the technique presented here allows the qualitative and semiquantitative determination of the direct transfer of any product through the placental barrier in the rat before it is transformed by maternal metabolism, permitting interpretation of results by simple comparison of the radioactive values found in fetal blood samples from the left and right uterine horns. The present technique is however not valid for quantification of the actual rate of rat placental transfer.

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References

1 Anand, R.S.; Sperling, M.A.; Ganguli, S.; Nathanielsz, P.W.: Bidirectional placental transfer of glucose and its turnover in fetal and maternal sheep. Pediat. Res. *13:* 783–787 (1979).

2 Bruce, N.W.: The distribution of blood flow to the reproductive organs of rats near term. J. Reprod. Fertil. *46:* 359–362 (1976).

3 Dancis, J.; Money, W.L.; Springer, D.; Levitz, M.: Transport of amino acids by placenta. Am. J. Obstet. Gynec. *101:* 820–829 (1968).

4 Eaton, B.M.; Yudilevich, D.L.: Uptake and asymmetric efflux of amino acids at maternal and fetal sides of placenta. Am. J. Physiol. *241:* C106–C112 (1981).

5 Elphick, M.C.; Hudson, D.G.; Hull, D.: Transfer of fatty acids across the rabbit placenta. J. Physiol. *252:* 29–42 (1975).

6 Freminet, A.; Leclerc, L.: Effect of fasting on glucose, lactate and alanine turnover in rats and guinea-pigs. Compar. Biochem. Physiol. *65B:* 363–367 (1980).

7 Greene, E.C.: Anatomy of the rat. Trans. Am. phil. Soc., vol. 27 (Mafner, New York 1968).

8 Herrera, E.; Knopp, R.H.; Freinkel, N.: Carbohydrate metabolism in pregnancy. VI. Plasma fuels, insulin, liver composition, gluconeogenesis, and nitrogen metabolism during late gestation in the fed and fasted rat. J. clin. Invest. *48:* 2260–2272 (1969).

9 Hodgson, J.C.; Mellor, D.J.; Field, A.C.: Rates of glucose production and utilization by the foetus in chronically catheterized sheep. Biochem. J. *186:* 739–747 (1980).

10 Hugget, A.S.G.; Nixon, D.A.: Use of glucose oxidase, peroxidase, and O-dianisidine in determination of blood and urinary glucose. Lancet *i:* 368–370 (1957).

11 Iglesias, R.: Efecto del tratamiento crónico con TRH sobre la función hipofisaria en la rata; thesis Universidad de Barcelona (1978).

12 Kastendieck, E.; Moll, W.: The placental transfer of lactate and bicarbonate in the guinea pig. Pflügers Arch. *370:* 165–171 (1977).

13 Kihlström, K.; Kihlström, J.E.: An improved technique for perfusion of the guinea pig placenta in situ giving viable conditions demonstrating by placental transport of amino acids (*L*- and *D*-alanine). Biol. Neonate *39:* 150–159 (1981).

14 Lemons, J.A.; Adcock, E.W. III; Jones, D., Jr.; Naughton, M.A.; Meschia, G.; Battaglia, F.C.: Umbilical uptake of amino acids in the unstressed fetal lamb. J. clin. Invest. *58:* 1428–1434 (1976).

15 Meschia, G.; Cotter, J.R.; Makowski, E.L.; Barron, D.H.: Simultaneous measurement of uterine and umbilical blood flows and oxygen uptakes. Q. Jl exp. Physiol. *52:* 1–18 (1967).

16 Money, W.L.; Dancis, J.: Technique for the in situ

study of placental transport in the pregnant guinea pig. Am. J. Obstet. Gynec. *80:* 209–214 (1960).
17 Rice, P.A.; Rourke, J.E.; Nesbitt, R.E.L., Jr.: In vitro perfusion studies of the human placenta. VI. Evidence against active glucose transport. Am. J. Obstet. Gynec. *133:* 649–655 (1979).
18 Rosso, P.; Kava, R.: Effects of food restriction on cardiac output and blood flow to the uterus and placenta in the pregnant rat. J. Nutr. *110:* 2350–2354 (1980).
19 Schneider, H.; Panigel, M.; Dancis, J.: Transfer across the perfused human placenta of antipyrine, sodium and leucine. Am. J. Obstet. Gynec. *114:* 822–828 (1972).
20 Smith, C.M.; Adcock, E.W. III; Teasdale, F.; Meschia, G.; Battaglia, F.C.: Placental amino acid uptake: tissue preparation, kinetics, and preincubation effect. Am. J. Physioi. *224:* 558–564 (1973).
21 Snedecor, G.W.: Statistical methods; 5th ed. (Iowa State University Press, Ames 1956).
22 Somogyi, M.: Determination of blood sugar. J. biol. Chem. *160:* 69–73 (1945).
23 Widdas, W.F.: Inability of diffusion to account for placental glucose transfer in sheep and consideration of the kinetics of a possible carrier transfer. J. Physiol. *118:* 23–39 (1952).
24 Williamson, D.H.: *L*-Alanine determination with alanine dehydrogenase; in Bergmeyer, Methods of enzymatic analysis, vol. 4, pp. 1679–1682 (Chemie & Academic Press, New York 1974).
25 Yudilevich, D.L.; Eaton, B.M.; Short, A.H.; Leichtweiss, H.-P.: Glucose carriers at maternal and fetal sides of the trophoblast in guinea pig placenta. Am. J. Physiol. *237:* C205–C212 (1979).

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