0031-3998/83/1709-0729$02.00/0
PEDIATRIC RESEARCH

Vol. 17, No. 9, 1983
Printed in U.S.A.

Metabolism of the Human Placenta Perfused *in Vitro*: Glucose Transfer and Utilization, O_2 Consumption, Lactate and Ammonia Production

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Summary

Oxygen consumption, glucose transfer and utilization, and lactate and ammonia release were studied in the human full term placenta using an *in vitro* perfusion technique. D-Glucose was the only substrate added to the perfusion fluid. On the maternal side, oxygen and glucose were taken up at a constant rate during perfusion. On the fetal side, a lower oxygen uptake was observed. Glucose was steadily released into the fetal perfusate as a result of the downhill concentration gradient established between maternal and fetal circulations. By contrast, lactate and ammonia release took place only into the maternal circulation and decreased rapidly during perfusion. No significant change in tissue glucose content was observed between the onset and the end of the experiment. Placental lactate and ammonia concentrations were shown to diminish significantly during perfusion.

Abbreviation

v-a, veno-arterial

The supply of oxygen, glucose, and amino acids to the fetus has often been studied without due regard to their rate of metabolism in the placenta. There are now several lines of evidence indicating that placental metabolism interferes with the transfer of certain endogenous molecules. During transfer across the perfused human placental lobule, an extensive conversion of cortisol to cortisone has been demonstrated (4). Furthermore the lack of glutamate transfer has been interpreted as being the result of its rapid uptake and degradation by the trophoblastic membrane (19).

The role of placental metabolism in relation to the supply of glucose and lactate to the fetus was studied recently in sheep. Placental tissue utilization of glucose seems to be implicated in the limitation of its materno-fetal transfer (22). Maternal lactate is transferred only in small amounts across the sheep cotyledon(11); however, this substrate is produced by the placenta and release into the umbilical circulation (1, 23).

The purpose of this study is to reexamine some aspects of transfer and metabolism in the perfused human placenta using only D-glucose as substrate. Oxygen consumption, glucose transfer and utilization, lactate and ammonia release were investigated to serve as a baseline for further studies with additional substrates.

MATERIALS AND METHODS

Perfusion technique. Human placentae from uncomplicated pregnancies were collected after vaginal delivery at term. The perfusion technique has been described in detail by Schneider *et al.* (18). A suitable lobule was selected for perfusion. The fetal vessels were canulated with polyethylene catheters. Two fine canulas were inserted directly into the intervillous space over the area blenched by perfusion of fetal circulation. The maternal outflow, emerging from openings in the decidual plate, was drained outside the perfusion chamber. The perfusion was started within 30 min after delivery. Fifteen minutes were allowed to wash out the blood from the circulations, after which the experiment lasted 45 min.

To allow for metabolic studies, changes in temperature control were introduced into the original description of the technique. The chamber containing the perfused lobule is surrounded by a water-jacket maintained at 37°C by a Heidolph thermomix T_{51} (West Germany). Maternal and fetal perfusion media pass through 8 ft of stainless steel coils immersed in a water bath at 37°C before entering the lobule. The volume of maternal outflow in the upper part of the perfusion chamber was kept minimal, in order to obtain representative samples of the perfusate emerging from the intervillous space. Earle's medium (NaCl, 6.8; KCl, 0.4; NaH_2PO_4, H_2O, 0.125; $MgSO_4$, 7 H_2O, 0.2; NaH_2CO_3, 1.54; and $CaCl_2$, 0.2 g/liter) was used as perfusion fluid in all experiments. D-Glucose concentration was 0.5 g/liter in the fetal medium and 1.5 g/liter in the maternal medium. Tritiated water (20 nCi/ml, C.E.A. Gif-surYvette, France) was added to maternal perfusion fluid. Maternal and fetal arterial perfusion fluids were continuously gassed with 95% O_2 + 5% CO_2. The venous perfusates were never recirculated. Flow rates were monitored with R 2 15 A Brooks flowmeters. Pressures were measured with mercury manometers (Boulitte, France). pH, P_{CO_2}, P_{O_2}, flow rates, and pressures of perfusion were monitored on fetal and maternal circulations, except the rate of maternal outflow.

Sampling and measurements. The experiment started when the maternal fluid containing tritiated water was connected to the perfusion circuit. Then, samples of perfusate were obtained every 15 min through a Y glass with stop-cocks, which were inserted on inflow and on outflow of fetal and maternal circuits near the perfusion chamber. For pH, P_{CO_2} and P_{O_2} measurements, samples were drawn with 1-ml disposable plastic syringes. The needles were inserted into rubber stoppers and kept in ice until the measurements, which were performed at the end of perfusion. For biochemical assays, 2 ml of perfusate were drawn and placed in ice. Aliquots of perfusate (1 ml) were immediately deproteinized with 0.5 ml of $ZnSO_4$ and 0.5 ml of $Ba(OH)_2$ solutions and were centrifuged for 5 min at 4000 × *g* and 4°C. The supernatants were stored at −20°C until glucose and lactate assays were performed. The remainder of perfusate was used for ammonia determination, which was carried out immediately after the perfusion, and for counting of tritium radioactivity.

Tissue samples weighing about 600 mg were excised from the placenta during the period of wash out of blood from the fetal circulation outside the area selected for perfusion and, at the end of the perfusion, inside the perfusion chamber from both the unperfused and perfused areas. For glucose and lactate measurements, three tissue fragments (100 mg each) from these samples

were stored at −20°C. The frozen tissues were homogenized at 4°C with a teflon homogenizer in 50 ml of the $Ba(OH)_2/ZnSO_4$ deproteinizing solution. For ammonia determination, three tissue fragments (100 mg each) from the above samples were quickly blotted, frozen in polypropylene tubes with liquid nitrogen and stored at −80°C until assayed. These frozen tissue fragments were homogenized in 50 ml of cooled bidistilled water. The biochemical assays were carried out on the supernatants obtained after centrifugation of the homogenates at 4°C and 4000 × *g* for 10 min. Glucose, lactate, and ammonia determinations were performed on perfusate and tissue samples. Glucose concentration was determined by the glucose oxidase method (10). Lactate concentration was measured by the lactate dehydrogenase method (7) using Sigma kit (precision of the assay: 18μmole/liter). Ammonia concentration was determined by a modification of the enzymatic method of Reichelt *et al.* (16) using Sigma kit (precision of the assay: 6 μmole/liter). This method is based on the reductive amination of α-ketoglutarate using glutamate dehydrogenase and NADH. The lack of interference of the endogenous placental substrate α-ketoglutarate in our assay was established in controls with α-ketoglutarate-free buffer. The metabolites were all found to remain stable in stored tissue at least for 8 days.

The tritium radioactivity of the perfusate samples was counted in a liquid scintillation spectrometer (Intertechnique, SL 3000). pH, P_{CO_2} and P_{O_2} measurements were done with a BMS 3 Mk2 blood gas analyzer (Radiometer, Copenhagen).

Calculations. The calculations were based on the veno-arterial concentration difference (C_v-C_a) in the perfusion fluid for each compound investigated. A positive veno-arterial difference indicates a release of substance from placental tissue, whereas a negative v-a difference means an uptake from the perfusate. The rates of release or uptake were calculated according to the relationship:

$$(C_v - C_a) \cdot Q \cdot \frac{1}{W}$$

where Q is the perfusion flow rate (ml/min) and W is the wet weight of the lobule (g). The algebric sum of fetal and maternal uptake or release of each compound represents its net metabolic rate in the placenta. The results were expressed in $\mu mole \cdot min^{-1} \cdot g^{-1}$ of tissue wet weight.

The O_2 content was calculated from the following relationship:

$$C = \frac{P_{O_2} \cdot 0.0239}{P_B - 47}$$

where P_{O_2} represents the oxygen partial pressure (mmHg), 0.0239 the solubility of O_2 in ml/ml of perfusate (5), P_B the barometric pressure (mmHg) and 47 the water vapor pressure (mmHg) at 37°C. Errors caused by physical gas leaks were evaluated on both circuits between the usual venous and arterial points of sampling by mean of two sham experiments in which a silicone membrane was substituted for the placental lobule. The leaks averaged 0.018 ± 0.02 μmole/min *i.e.*, 0.007 $\mu mole \cdot min^{-1} \cdot g^{-1}$ when expressed as a function of the mean cotyledon weight (23.6 g). These leaks were taken into account in the calculation of O_2 consumption.

For the compounds transferred from one circulation to the other such as glucose and tritiated water, the clearance (Cl) was calculated using the following relationship:

$$Cl = \frac{C_{Fv} - C_{Fa}}{C_{ma} - C_{Fa}} \cdot Q_F$$

where C_{Fv} is the fetal venous concentration, C_{Fa} the fetal arterial concentration, C_{Ma} the maternal arterial concentration of the substances studied, and Q_F the fetal flow rate.

RESULTS

The parameters of perfusion monitored in these experiments are given in Table 1. pH, P_{CO_2}, P_{O_2}, arterial pressures, and flow rates in the maternal and fetal circulations remained stable during the entire course of perfusion (data not shown). Oxygen, glucose, lactate, and ammonia uptake from and release into fetal and maternal perfusates measured in a single experiment are presented in Figure 1. The greatest uptake was observed for maternal glucose. Oxygen was taken up at a lower rate on fetal side than on the maternal side. Glucose was released in noticeable amounts into the fetal perfusate whereas lactate and ammonia remained undetectable. These two molecules appeared in the maternal perfusate, lactate release exceeding a thousandfold that of ammonia.

Mean values of uptake and release of oxygen and glucose are presented in Table 2. Oxygen was taken up from fetal and maternal circulations in the absence of a transplacental P_{O_2} gradient between arterial perfusion media. The total O_2 consumption of the perfused tissue amounted 0.30 ± 0.04 $\mu mole \cdot min^{-1} \cdot g^{-1}$. A large uptake of glucose 0.74 ± 0.05 $\mu mole \cdot min^{-1} \cdot g^{-1}$ was observed from the maternal circulation as a result of its transfer to the fetal circulation and its utilization by placental tissue. The glucose transfer rate averaged 0.31 ± 0.09 $\mu mole \cdot min^{-1} \cdot g^{-1}$. The mean clearance of glucose in these experiments was 1.51 ± 0.31 ml/min per lobule and that of tritiated water 2.30 ± 0.34 ml/min per lobule; thus, the ratio of glucose clearance to tritiated water clearance was 50 ± 13%.

Lactate and ammonia release rates, determined in the same set of perfusion studies, are plotted as a function of time in Figure 2. As already shown in Figure 1, the fetal efflux of lactate was not significant, the concentrations detected in the fetal venous perfusate being in the range of precision of the assay. On the maternal side, its rate of efflux was high at the onset of perfusion but decreased rapidly. In six experiments and during the 60 min of perfusion, the decreased rate of release of lactate into the maternal circulation in relation to time was found to be significant by analysis of linear regression ($r = 0.46$, $P < 0.05$). Ammonia followed a similar pattern in fetal and maternal outflows ($r = 0.43$, $P < 0.05$, three experiments). Lactate and ammonia release fell by 47% and 99%, respectively between 15 and 60 min of perfusion. After 60 min of perfusion, the rate of appearance of lactate in the maternal perfusate was still 0.34 ± 0.08 $\mu mole \cdot min^{-1} \cdot g^{-1}$ and that of ammonia was negligible. One experiment stands out as an exception: wherein both lactate and ammonia on the maternal side rose after a significant decrease. This experiment was included in the statistical analysis.

Glucose, lactate, and ammonia concentrations were also measured in tissues (Fig. 3). No significant difference could be demonstrated in glucose concentration between tissue fragments taken at the beginning of the period of wash out of blood vessels and unperfused tissue, and perfused tissue fragments taken at the end of the experiment. Interestingly, the concentration observed in perfused tissue is similar to that employed in the fetal perfusion medium but not to that of the maternal one. As compared to the initial value, lactate concentration in the tissue was shown to be significantly reduced ($p < 0.001$) during perfusion. Similarly, placental ammonia concentration was decreased both in the unperfused ($p < 0.002$) and the perfused areas ($p < 0.01$).

Table 1. *Parameters of perfusion in the arterial fetal and maternal fluids*[1]

	pH	P_{CO_2}	P_{O_2}	Pa	Qa	W
Maternal	7.42 ± 0.03	23.7 ± 2.3	394 ± 9.5	40.5 ± 10.7	22.8 ± 0.44	23.6 ± 1.8
Fetal	7.41 ± 0.03	23.5 ± 1.7	391 ± 6.5	25.5 ± 3.1	10.2 ± 0.28	

[1] P_{CO_2}, CO_2 partial pressure (mmHg); P_{O_2}, O_2 partial pressure (mmHg); Pa, arterial pressure (mmHg); Qa, flow rate of the arterial perfusion medium (ml/min); and W, cotyledon wet weight (g). Results are means ± S.E.M. of six separate experiments.

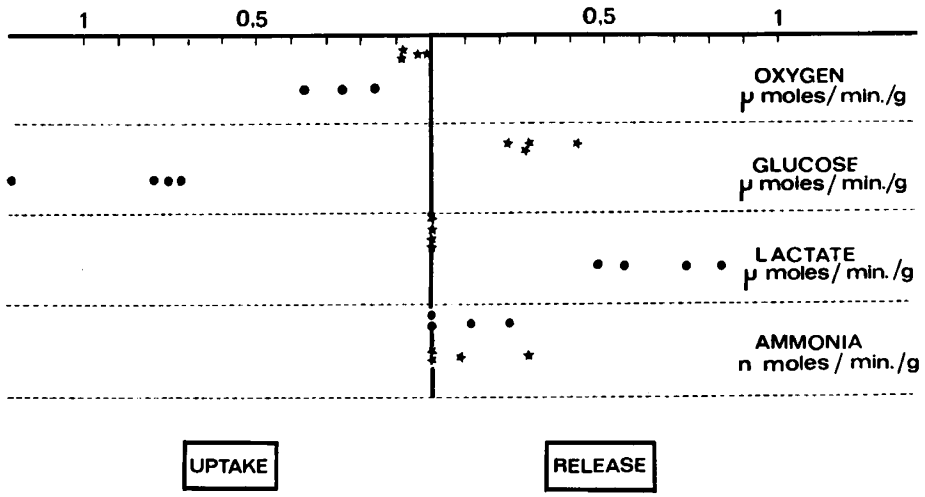

Fig. 1. Uptake and release of oxygen, glucose, lactate, and ammonia in one single perfusion. Uptake and release of substances refers to fetal (★) and maternal (●) circulations. Each point represents one measurement.

Table 2. *Uptake or release and net consumption of oxygen and glucose*[1]

Substrates (μmole/min/g)	Rates of uptake or release		Net consumption
	Fetal side	Maternal side	
Oxygen	−0.09 ± 0.01	−0.21 ± 0.03	−0.30 ± 0.04
Glucose	+0.31 ± 0.09	−0.74 ± 0.05	−0.43 ± 0.07

[1] Results are expressed in μmole/min/g. Values are means ± S.E.M. of six experiments. (+) indicates appearance and (−) disappearance of substances in the perfusion fluid.

DISCUSSION

In many tissues, glucose is considered to be one of the major substrates for energetic requirements. Its metabolism is closely related to O_2 consumption. In placental tissue, O_2 consumption has been studied *in vitro* either by incubation of slices (6, 24) or by perfusion (15). The values reported in these studies ranged between 0.13–0.21 $\mu mole \cdot min^{-1} \cdot g^{-1}$ placental wet weight. As was shown in a previous study (3) in which whole human fresh blood was used at physiologic hematocrit and Po_2, the O_2 consumption can reach 0.49 $\mu mole \cdot min^{-1} \cdot g^{-1}$. This value is in good agreement with those observed in sheep (2) and in guinea-pigs (12). In the present experiments, the difficulty of obtaining human fetal and adult blood in sufficient amount led us to use a buffered saline solution equilibrated with 95% O_2 + 5% CO_2 as perfusion fluid in both circulations. This solution supplies a large though not maximal amount of O_2 to the perfused tissue. Under these conditions placental O_2 consumption averaged 0.30 $\mu mole \cdot min^{-1} \cdot g^{-1}$ wet weight. This value was corrected for errors caused by physical leakage, which represents only 0.2% of the O_2 consumption. It is greater than that observed in the slice incubation technique but remains below the rate expected *in vivo*, 0.38 $\mu mole \cdot min^{-1} \cdot g^{-1}$ (3). This oxygen consumption may represent a situation of mild hypoxia in comparison to *in vivo* conditions.

The high placental glucose utilization rate (0.43 ± 0.07 $\mu mole \cdot min^{-1} \cdot g^{-1}$) as well as O_2 consumption are indicative of active metabolism by the perfused tissue. The mean glucose utilization rate greatly exceeds that reported with the slice incubation technique (8, 17) and perfusion of the whole placenta (9, 14). Nevertheless this value of glucose utilization is in the range of those observed in dual perfusion of isolated lobule (20). Almost 60% of the glucose taken up from maternal circulation stays in the tissue and is presumably utilized by the placenta, the remainder being transferred to the fetus. This also accords well with the observations reported in perfusion of human placenta by Schneider *et al.* (20) and in the sheep by Simmons *et al.* (21).

In contrast to the steady values of uptake and release observed for O_2 and glucose in fetal and maternal circulations, maternal lactate release and maternal and fetal ammonia release were shown to be time-related. To our knowledge these observations have not been reported so far. The high levels of lactate and ammonia at the start of perfusion probably reflect the degradation of endogenous substrates during the period of ischemia and anoxia, which occurs before the perfusion. The subsequent decrease in concentration of these metabolites as perfusion proceeds is indicative of their washout from blood or from blood and tissue. But the observed release of lactate cannot be attributed entirely to wash out and may reflect glucose utilization, at least in part. In the absence of a precursor other than glucose, lactate concentration in maternal outflow dropped to only 50% of its initial value after 60 min of perfusion. In contrast, ammonia, whose precursors were not supplied by the perfusion medium exhibited a rapid decrease in concentration in the outflow and became extremely low after 30 min of perfusion.

Although glucose tissue concentrations were maintained until the end of the perfusion, both lactate and ammonia tissue concentrations were reduced significantly. This pattern of lowered concentrations of lactate and ammonia in perfused tissue agrees well with that of their release into the outflow. The wash out due to perfusion seems able to restore more physiologic tissue levels of these metabolites.

These observations make it difficult to compare our *in vitro* absolute figures of lactate and ammonia release to those observed in other studies unless the start of perfusion and the time of sampling are provided. It is equally difficult to compare our results to findings obtained with chronic sheep preparation in which metabolic fuels other than glucose are supplied to the placenta in physiologic amounts. Nevertheless, relative comparisons are possible. Lactate release represents about 40% of glucose utilized, a value in accordance with that found by Nesbitt *et al.* (14) in perfusion studies. As regards to the absolute figures, it is worth noting that the absence of ammonia in the perfusate at the end of the experiment is in contrast to the net production observed by Holzman *et al.* (8) in slice incubations.

The human placenta seems to be characterized by a high glucose utilization rate. The amount of glucose contributing to aerobic metabolism can be estimated from the glucose/oxygen ratio defined by Morriss *et al.* (13): $6 \cdot \Delta G/\Delta O_2$. Considering that all the oxygen is utilized for glucose oxidation, this ratio should be equal to 1. Glucose utilization estimated from $\Delta O_2/6$ averaged 0.05 ± 0.01 $\mu mole \cdot min^{-1} \cdot g^{-1}$. On the other hand, assuming a complete transformation of glucose into lactate, the rate of glucose utilization calculated from $\Delta L/2$ approximates 0.17 ± 0.04 $\mu mole \cdot min^{-1} \cdot g^{-1}$; therefore, O_2 consumption and lactate production represent 20 ± 4% and 38 ± 5% respectively, of glucose utilization. These results show that some of the glucose may also contribute to energy requirements other than those of oxidative metabolism

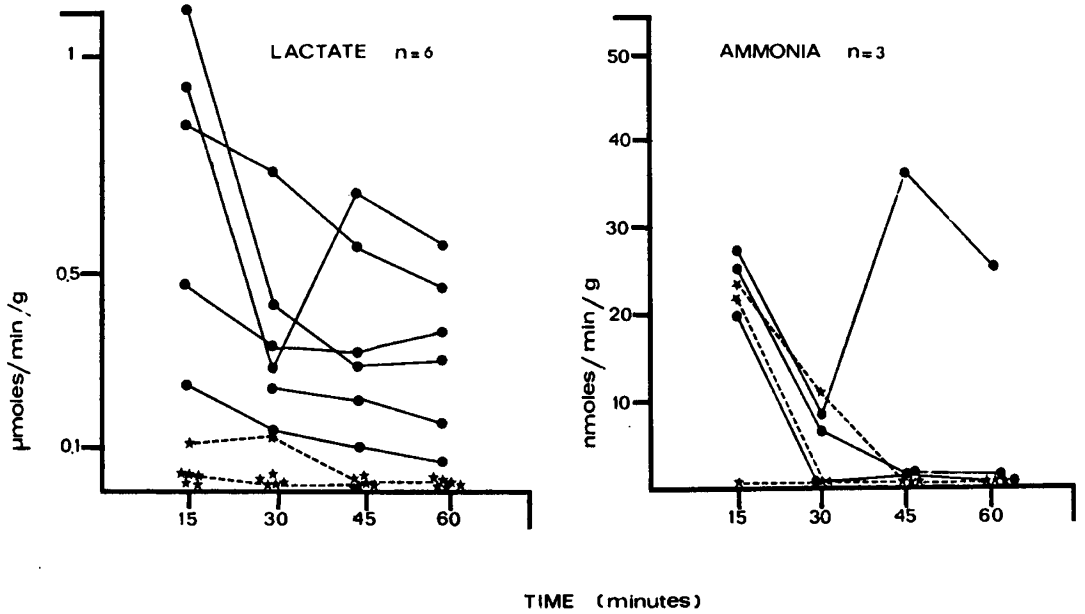

Fig. 2. Lactate and ammonia release into the perfusion fluids. Each point represents a single measurement. Six experiments were performed. Ammonia was determined in three experiments. (★) fetal perfusate and (●) maternal perfusate.

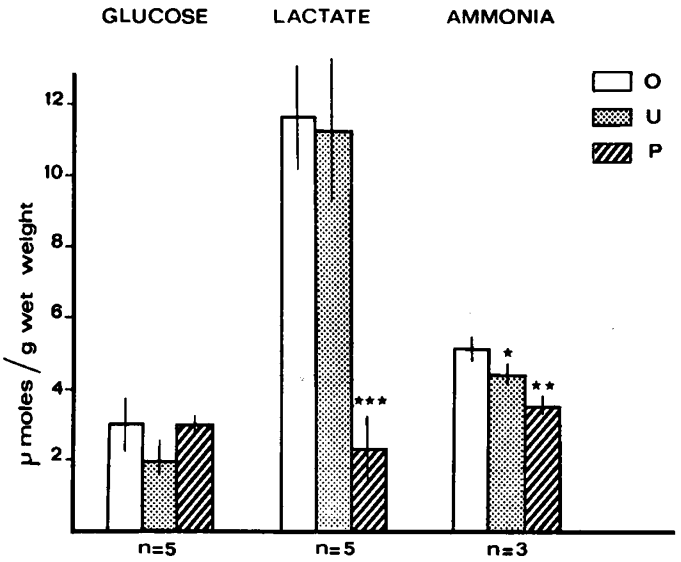

Fig. 3. Glucose, lactate and ammonia concentrations in placental tissue. O, tissue collected 30 min after delivery at the beginning of the period of blood wash out; U, tissue collected at the end of perfusion from an unperfused area; and P, tissue collected at the end of perfusion from the perfused area. *Vertical bars* indicate mean ± S.E.M. of six experiments, (***), $P < 0.001$ as compared to the initial concentration (O); (**), $P < 0.01$; and (*), $P < 0.02$. Paired Student's test was used for the statistical analysis.

in the human placenta. Further experiments would seem necessary to assess the amount of glucose contributing to lactate production and to determine which metabolic pathways account for the unexplained glucose utilization.

REFERENCES AND NOTES

1. Burd, L. I., Jones, M. D., Simmons, M. A., Makowski, E. L., Meschia, G., and Battaglia, F. C.: Placental production and utilization of lactate and pyruvate. Nature, *254:* 710 (1975).
2. Campbell, A. G., Dawes, G. S., Fishman, A. P., Hyman, A. I., and James, G. B.: The oxygen consumption of the placenta and foetal membranes in the sheep. J. Physiol. (London), *182:* 439 (1966).
3. Challier, J. C., Schneider, H., and Dancis, J.: *In vitro* perfusion of human placenta. V. Oxygen consumption, Am. J. Obstet. Gynecol., *126:* 261 (1976).
4. Dancis, J., Jansens, V., Levitz, M., and Rosner, W.: Effect of protein binding on transfer and metabolism of cortisol in perfused human placenta. J. Clin. Endoc. Metab., *46:* 863 (1978).
5. Dixon, M.: Manometric methods Ed. 2. (Cambridge University Press, Cambridge, 1943).
6. Friedman, E. A. and Sachtleben, M. R.: Placental oxygen consumption *in vitro*. Baseline studies. Am. J. Obstet. Gynecol., *79:* 1058 (1960).
7. Henry, R. J.: Principles and techniques. In: Clinical Chemistry. pp 664–666 (Harper and Row, New York, 1968).
8. Holzman, I. R., Philips, A. F., and Battaglia, F. C.: Glucose metabolism, lactate and ammonia production by the human placenta *in vitro*. Pediatr. Res., *13:* 117 (1979).
9. Howard, J. M. and Krantz, K. E.: Transfer and use of glucose in the human placenta during *in vitro* perfusion and the associated effects of oxytocin and papaverine. Am. J. Obstet. Gynecol., *98:* 445 (1967).
10. Huggett, A. S. G. and Nixon, D. A.: Use of glucose oxidase peroxidase and O-dianisidine in determination of blood and urinary glucose. Lancet, *2:* 368 (1957).
11. Kastendieck, E., Kunzel, W., and Kurz, C. S.: Placental clearance of lactate and bicarbonate in sheep. Gynecol. Obstet. Invest., *10:* 9 (1979).
12. Leichtweiss, H. P. and Schröder, H.: Dual perfusion of the isolated guinea-pig placenta. Placenta, *Suppl. 2:* 119 (1981).
13. Morriss, F. H., Makowski, E. L., Meschia, G., and Battaglia, F. C.: The glucose/oxygen quotient of the human term fetus. Biol. Neonate, *25:* 44 (1975).
14. Nesbitt, R. E. L., Rice, P. A., and Rourke, J. E.: *In vitro* perfusion studies of the human placenta. III. The relationships between glucose utilization rates and concentration. Gynecol. Invest., *4:* 243 (1973).
15. Nyberg, R. and Westin, B.: The influence of oxygen tension and some drugs on human placental vessels. Acta Physiol. Scand., *39:* 216 (1957).
16. Reichelt, K. L., Kvamme, E., and Tveit, B.: The enzymatic determination of ammonia in blood and tissues. Scand. J. Clin. Lab. Invest., *16:* 433 (1964).
17. Sakurai, T., Tagaki, H., and Hosoya, N.: Metabolic pathways of glucose in human placenta. Changes with gestation and with added 17β-estradiol. Am. J. Obstet. Gynecol., *105:* 1044 (1969).
18. Schneider, H., Panigel, M., and Dancis, J.: Transfer across the perfused human placenta of antipyrine sodium and leucine. Am. J. Obstet. Gynecol., *114:* 822 (1972).
19. Schneider, H., Möhlen, K., H., Challier, J. C., and Dancis, J.: Transfer of glutamic acid across the human placenta perfused *in vitro*. Brit. J. of Obstet. Gynaecol., *86:* 299 (1979).
20. Schneider, H., Challier, J. C., and Dancis, J.: Transfer and metabolism of glucose and lactate in the human placenta studied by a perfusion system *in vitro*. Placenta, *Suppl. 2:* 129 (1981).
21. Simmons, M. A., Burd, L. J., Lemons, J. A., Jones, M. D., Schreiner, R. L., Meschia, G., and Battaglia, F. C.: Placental utilization and conversion of glucose *in vivo*. Pediatr. Res., *9:* 279 (1975).
22. Simmons, M. A. and Battaglia, F. C.: Placental transfer of glucose. J. Dev. Physiol., *1:* 227 (1979).
23. Sparks, J. W., Hay, W. W., Bonds, D., Meschia, G., and Battaglia, F. C.: Simultaneous measurements of lactate turnover rate and umbilical lactate uptake in the fetal lamb. J. Clin. Invest., (in press) (1981).
24. Villee, C. A.: Metabolism of human placenta *in vitro*. J. Biol. Chem., *205:* 113 (1953).

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26. Received for publication November 4, 1981.
27. Accepted for publication November 19, 1982.