Original articles

J. Perinat. Med.
10 (1982) 147

The metabolism of the isolated artificially perfused guinea pig placenta
I. Excretion of hydrogen ions, ammonia, carbon dioxide and lactate, and the consumption of oxygen and glucose

M. H. Carstensen, H. P. Leichtweiß, H. Schröder

Universitätsfrauenklinik Eppendorf, Abteilung für experimentelle Gynäkologie, 2000 Hamburg-Eppendorf

1 Introduction

The hemochorial guinea pig placenta serves as a model for numerous investigations on transplacental transport. KASTENDIECK and MOLL [7] demonstrate, using an in situ perfusion technique on the fetal side, that the transport of lactate and bicarbonate across the placenta is proportional to the concentration differences. In a recent paper [13] the same investigators suggest a stereospecific, carrier mediated process (facilitated diffusion) for the transfer of lactate. For the human term placenta [11] and for the guinea pig placenta [10] our group could show the carrier system for lactate to be located in both membranes of the trophoblast (maternal and fetal side). LONGO et al. [12] evaluate the carbon dioxide transport through the epitheliochorial placenta of sheep: carbon dioxide mainly diffuses as gas and only to a small amount as bicarbonate. In these investigations the transport through the placenta is determined without measuring simultaneously the metabolism of the placenta itself. It is well known, that the human placenta produces lactate and carbon dioxide [3, 5]. For the guinea pig placenta, the quality and quantity of metabolites are unknown. **The present study was designed to measure simultaneously the production of hydrogen ions, ammonia, carbon dioxide, lactate and the consumption of oxygen and glucose in the isolated and artificially perfused guinea pig placenta.**

Curriculum vitae

MARTIN H. CARSTENSEN *was born in 1946 and obtained his medical degree in 1975. He worked from 1975 until now in the Universitätsfrauenklinik Eppendorf in Hamburg. Main research interests: Metabolism and transport systems in the placenta of human and guinea pig, beta mimetics, prolactin and sterility.*

2 Methods

Guinea pig dams at 50–60 days of gestation are anesthetized with 10 mg diazepam (Valium®, ROCHE) intraperitoneally and 50–100 mg ketamine (Ketanest®, PARKE DAVIS) intramuscularly. The dam is placed supine in a Ringer solution bath of 37 °C. After opening of the abdomen, nifedipine (Adalat®, BAYER) is given locally to relax uterine muscles. Uterotomy is performed at the antimesometrial border. Then two rings of 5 cm diameters are clamped together around the placenta, squeezing the uterine muscles and vessels, except those associated with the placenta [8]. The fetal side is perfused after cannulating one umbilical artery and the umbilical vein. The maternal

0300-5577/82/0010-0147$02.00

vessels are cannulated and perfused in the same manner. Remaining maternal vessels are ligated and cut. The uterine muscles around the outer edge of the ring are cut away, too. The completely isolated placenta is placed in a 37 °C warm organ bath. Both – the maternal and fetal side – are perfused with TC 199 tissue culture medium (Difco), to which 1 g/l bovine albumine has been added. This balanced salt solution contains all the essential amino acids. The fluid is saturated at 37 °C with 95% O_2 and 5% CO_2. A pH of 7.4 is maintained by adding 10–20 mmol/l bicarbonate. Perfusion flow rates can be changed stepwise from 1.6 to 6.2 ml/min by means of glass syringe pumps. Perfusion pressures range from 10–12 mm Hg on fetal and from 10–15 mm Hg on maternal side. Further details have been described earlier [11, 15, 18]. The pH, pO_2 and pCO_2 are determined with an automatic blood gas analyzer (CORNING 175). The concentration of hydrogen ions is calculated from the pH and a curve of titration, established with an aliquot of the perfusion fluid, which is equilibrated with carbogen and contains no ammonia. The excretion rate of hydrogen ions is defined as the equivalent of acid (HCl), necessary to decrease the pH of this solution to the pH values of the venous samples. The excretion rates thus do not include the hydrogen ions buffered by ammonia and other metabolites. The rate E (CO_2) of excretion of CO_2 is calculated by the equation:

$$E(CO_2) = \Delta pCO_2 \times \alpha' \times q \times g^{-1} \quad (10^{-6}\ \text{mol} \times g^{-1} \times \text{min}^{-1}) \qquad [1].$$

The rate U (O_2) of utilization of oxygen is determined by the equation:

$$U(O_2) = \Delta pO_2 \times \beta' \times q \times g^{-1} \quad (10^{-6}\ \text{mol} \times g^{-1} \times \text{min}^{-1}) \qquad [2].$$

q = perfusion flow (ml × min^{-1}),
g = placental wet weight (g)
ΔpCO_2 = venous-arterial difference of CO_2 tension (mm Hg)
ΔpO_2 = arterial-venous difference of O_2 tension (mm Hg)
α' = .03 × 10^{-6} mol × ml^{-1} × mm Hg^{-1}
β' = .0015 × 10^{-6} mol × ml^{-1} × $mmHg^{-1}$

Diffusional exchange of O_2 and CO_2 across the walls of catheters, tubes, and the surface of the placenta occur and will influence the arterial-venous differences. Therefore we determined as "arterial tension" the O_2- and CO_2 partial pressures in the perfusion fluid in the tip of the arterial cannulas. Diffusional exchanges in the short venous catheters are negligible. The placenta itself is covered with a rubber sheet.

The concentration of lactate is measured by a standard enzymatic method (Monotest Lactate, BOEHRINGER MANNHEIM) or with the Lactate Analyzer 640 (HOFFMANN LA ROCHE). The concentration of ammonia is determined using a special ammonia electrode (PHILIPS). The excretion rates can be calculated from their a–v differences, the perfusion flow and the placental weight. The utilization of glucose is determined from the a–v difference of the glucose concentrations, both the uterine and umbilical vessels (Glucokinase method, BECKMANN), the perfusion flow and the placental weight.

Thin layer chromatography is used for the discrimination between labelled L-lactate and pyruvate. Polygram CEL 400 plates, 0.1 mm (MACHEREY-NAGEL, DÜREN) were developed with 1 M ammonium/isobutyrate (10:3 v/v). Radioactive bands are detected with a BERTHOLD model LB 2723 scanner.

Excretion and utilization rates throughout this paper are given as 10^{-6} mol × g^{-1} × min^{-1}.

3 Results

The artificially perfused guinea pig placentas produce in steady state experiments hydrogen ions, ammonia, lactate and carbon dioxide for at least 90 minutes in rather constant amounts. Fig. 1 demonstrates a typical experiment. The excretion of hydrogen ions on both the maternal and fetal side ranges from .29 to .37, the excretion of ammonia from .38 to .44, of carbon dioxide from .60 to .74, and of lactate from .59 to .73. The excretion of these metabolites remains essentially unchanged in this placenta for at least 120 minutes.

In spite of constant experimental conditions the values show a large inter- and a minor intraplacen-

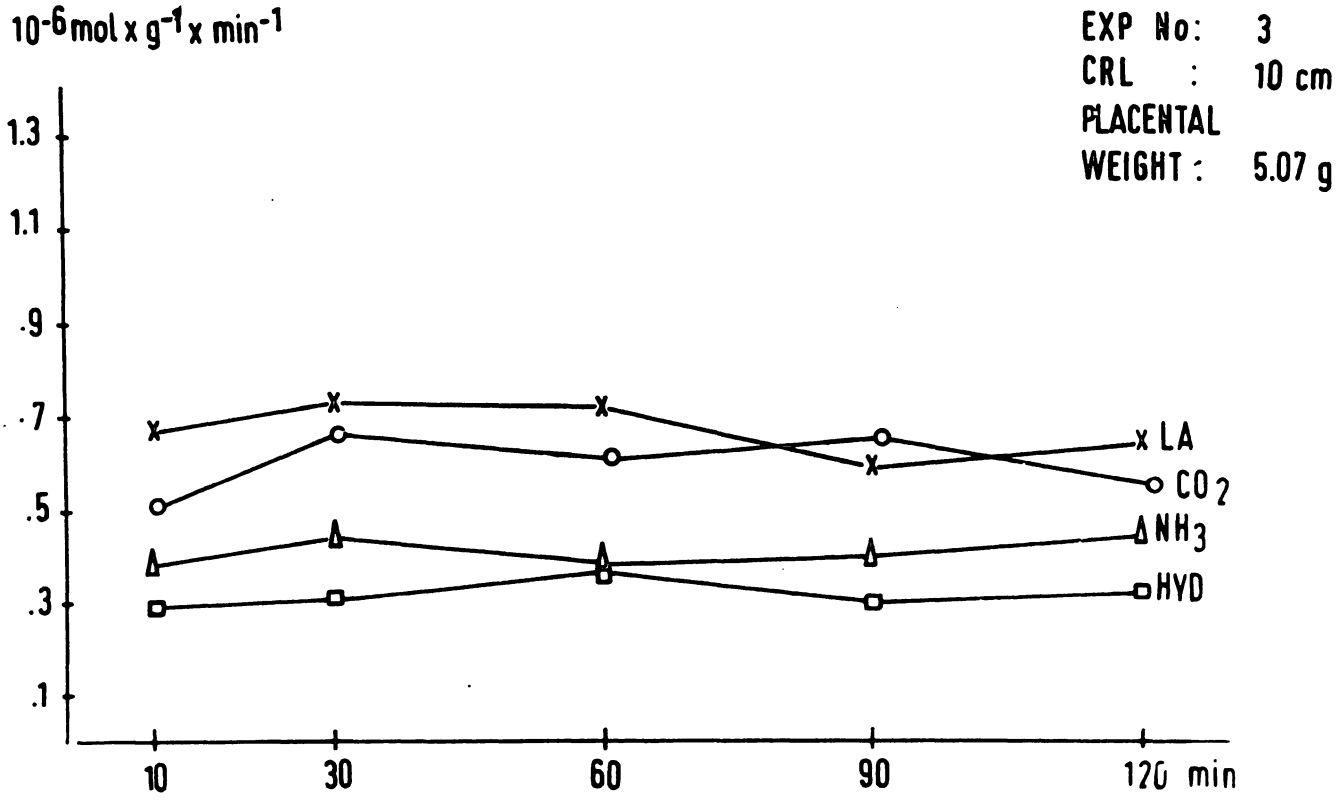

Fig. 1. Excretion rates of a dually perfused placenta. Ordinate: excretion rates of lactate (LA), CO_2, ammonia (NH_3) and hydrogen ions (HYD) as 10^{-6} mol × g^{-1} × min^{-1}. Abscissa: perfusion time.

Tab. I. Excretion of hydrogen ions, ammonia, carbon dioxide and lactate. Mean and SD of 22 placentas. (N) = total no. of measurements (1–5 measurements per placenta).

	(10^{-6} mol × g^{-1} × min^{-1})	
Hydrogen ions	.46 ± .22	(83)
Ammonia	.33 ± .14	(47)
Carbon dioxide	.65 ± .37	(45)
Lactate	.83 ± .54	(49)

tal variability. In Tab. I the means and standard deviations of all measurements within 90 min from 22 placentas are summarized.

In the following experiments the flow rates are changed stepwise (Tab. II). When the total flow is increased from 4.8 to 6.4 to 9.4 ml/min the production of hydrogen ions ($p < .01$) and of lactate ($p < .05$) increases significantly, while the excretion of CO_2 rises only slightly.

In 5 placentas we determined simultaneously the consumption of glucose and oxygen together with the production of hydrogen ions, ammonia, carbon dioxide and lactate (Tab. III).

Tab. II. Excretion of hydrogen ions, carbon dioxide and lactate at different flow rates. Mean and SD of 7 (Q_s = 4.8) and 6 placentas (Q_s = 9.4), compared to 22 placentas, perfused with the standard flow rate (Q_s) of 6.4 ml × min^{-1}. (N) = total no. of measurements (1–5 measurements per placenta).

(10^{-6} mol × g^{-1} × min^{-1})				
Hydrogen ions	Carbon dioxide	Lactate	Q_s
.33 ± .12 (34)	.46 ± .22 (14)	.60 ± .38 (12)	4.8
.46 ± .22 (79)	.65 ± .37 (45)	.83 ± .54 (49)	6.4
.59 ± .24 (15)	.56 ± .20 (6)	1.37 ± .68 (6)	9.4

Q_s = Sum of maternal and fetal flow (ml × min^{-1})

At constant flow rates the placenta utilizes .51 ± .11 oxygen and .35 ± .25 glucose. The excretion of hydrogen ions in these placentas amounts to .22 ± .09, the excretion of ammonia is .16 ± .09, of carbon dioxide .53 ± .21 and of lactate .53 ± .13. In all experiments described above the arterial glucose concentration is 5.5 mmol/l and the oxygen partial pressure more than 450 mmHg, when the fluid enters the placenta.

Tab. III. Utilization of oxygen and glucose, excretion of hydrogen ions, ammonia, carbon dioxide and lactate. Mean and SD of 5 placentas. (N) = total no. of measurements (4–7 measurements per placenta).

(10^{-6} mol × g^{-1} × min^{-1})

Utilization		Excretion			
Oxygen	Glucose	Hydrogen ions	Ammonia	Carbon dioxide	Lactate
.51 ± .11 (30)	.35 ± .25 (22)	.22 ± .09 (26)	.16 ± .09 (31)	.53 ± .21 (30)	.53 ± .13 (33)

In the following experiments the concentration of glucose in the perfusion fluid is changed in different sequences from 5.5 to 16.7 mmol/l, whereas the flow rate is kept constant at 3.2 ml/min on both sides (Tab. IV).
At the beginning and at the end of each experiment the physiological concentration of 5.5 mmol/l is used. The production of lactate shows no significant dependence on the glucose concentration.
In another set of experiments the arterial oxygen partial pressure is varied between 50, 145–150 and 400–500 mmHg (Tab. V). Flow rates are constant on both sides at 3.2 ml/min.

Thus a reduction of the oxygen tension in the perfusion fluid does not result in an increase of production of lactate.
We investigated the reduction of pyruvate to lactate under conditions with $pO_2 > 450$ mmHg (fig 2). Fig 2 shows a thin layer chromatography of 14 C-pyruvate, which has been injected into the fetal artery (donor). After one single passage through the placenta the pyruvate is reduced to lactate (maternal and fetal vein). A reference scan with a mixture of ^{14}C-L-lactate and ^{3}H-pyruvate is at the bottom of figure 2.

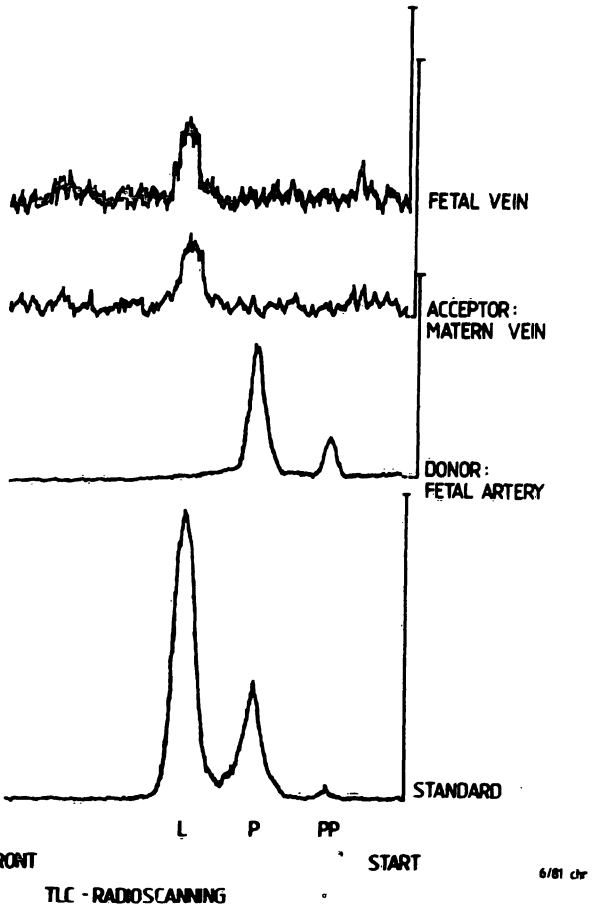

Fig. 2. Thin layer chromatography of ^{14}C-pyruvate, injected into the fetal artery (donor), and of fetal and maternal venous fluid. Bottom: reference scan of ^{3}H-L-lactate (L) and ^{14}C-pyruvate (P). PP = para-pyruvate.

Tab. IV. Excretion of lactate at different glucose concentrations in the perfusion fluid. Mean and SD of 4 placentas. (N) = total no. of measurements (4–6 measurements per placenta).

Glucose (mmol × l^{-1})	5.5	11.1	16.7	5.5
Lactate (10^{-6} mol × g^{-1} × min^{-1})	.75 ± .19 (22)	.85 ± .16 (23)	.78 ± .18 (22)	.73 ± .18 (22)

Tab. V. Excretion of lactate at different oxygen tension in the perfusion fluid. Mean and SD of 4 placentas. (N) = total no. of measurements (6–8 measurements per placenta).

pO_2 (mmHg)	400–500	145–150	< 50
Lactate (10^{-6} mol × g^{-1} × min^{-1})	.66 ± .09 (24)	.61 ± .16 (27)	.56 ± .19 (29)

4 Discussion

HOLZMAN et al. [5] show the ability of the human term placenta to produce in vitro lactate and ammonia. They do not find a correlation between the utilization of glucose and the production of lactate and ammonia. Similar results are reported by DIJKHUIZEN et al. [3], who conclude, that lactate must be the endproduct of glycolysis in the placenta. Comparing our data with the results from HOLZMAN et al. [5] we find 5–7 times higher values in the artificially perfused guinea pig placenta (Tab. I). This may be due to species differences and probably to the fact, that the metabolic efficiency of a perfused organ is greater than that of a tissue culture. The metabolism of the isolated placenta remains stable for a period of at least 90–120 minutes (Fig. 1).

Increased perfusion flow rates cause a significant increase of the excretion of hydrogen ions ($p < .01$) and lactate ($p < .05$), while the excretion of carbon dioxide only rises slightly. This may be caused by perfusing additional areas at higher flow rates. Secondly the convection of substrata in the tissue should be increased at higher flow rates. The production of lactate, however, is not influenced by the glucose concentration in the perfusion fluid.

The rate of the glucose utilization in the guinea pig placenta exceeds that of human placental tissue in vitro [5] and that of the perfused human placenta [6, 9, 17]. Our data agree with the results reported recently by SCHNEIDER et al. [14].

The relative high rate of lactate production is possibly not only attributable to the utilization of glucose, but also to the metabolism of amino acids. To answer this question the important parameters of energy metabolism (consumption of glucose and oxygen and production of lactate and carbon dioxide) should be balanced. The steady state data of 5 placentas are given in Tab. III. If $.53 \times 10^{-6}$ mol lactate and $.53 \times 10^{-6}$ mol CO_2 are excreted and attributed completely to the metabolism of glucose, $.35 \times 10^{-6}$ mol glucose should be utilized. These data (Tab. III) indicate, that **the production of lactate can be explained solely by the catabolism of glucose without catabolism of amino acids.** The high rate of production of lactate and the consumption of glucose are characteristic for the placenta in different species [1, 2, 16, 17]. In our experiments the equivalent of 24% of the utilized glucose are recovered as CO_2. This agrees with the results of the human term placenta [14], where this percentage is about 20%. In the sheep placenta the corresponding value lies with 40% essentially higher [16].

Thus the guinea pig placenta seems to cover its energy requirement mainly by means of the anaerobic glycolysis. The excretion of lactate does not increase, when the oxygen tension is reduced below 50 mm Hg. The oxygen supplied to the placenta then is less than 1/3 of the amount consumed under control conditions. Probably the lactate production is maximal in the placental tissue under normal conditions.

Pyruvate is reduced completely into lactate by a single passage through the placenta (Fig. 2). As can be expected the inverse reaction of the LDH cannot be demonstrated. Both – maternal and fetal injections of lactate – remain unchanged after perfusion through the placenta.

The ratio of dissociated lactic acid/undissociated lactate is about 3000/1 for a pH = 7.4 [7]. Our findings suggest that the produced lactate is buffered by the buffer systems, contained in the perfusion fluid (bicarbonate, phosphate and albumine) and by ammonia, which is produced by the placental tissue. The amount of hydrogen ions, which is buffered by ammonia is 42% of the total excretion of H^+-ions (hydrogen ions + ammonia, Tab. I). The production of ammonia has been published for the human and the sheep placenta [5, 4]. **The authors suggest ammonia to be a common product of placental metabolism,** because ureopoiesis is absent. This suggestion is confirmed by our results, because the isolated and artificially perfused guinea pig placenta produces ammonia, too. The source of the excreted ammonia could be glutamine, which is the only amino acid reported to be utilized by the human term placenta in tissue culture [5].

Summary

In the isolated, perfused guinea pig placenta glucose seems to be a major nutrient of energy metabolism, because the excreted amounts of carbon dioxide and lactate can be explained solely by the catabolism of glucose.
In steady state experiments hydrogen ions, ammonia, lactate and carbon dioxide are excreted for at least 90 minutes in rather constant amounts.
The production of lactate shows no significant dependence on the glucose concentration in the perfusion fluid.
The guinea pig placenta seems to cover its energy requirement mainly by means of anaerobic glycolysis.
Of the utilized glucose 76% are metabolized anaerobically.
The placenta produces significant quantities of lactate, although it is well oxygenated.
A reduction of the oxygen tension in the perfusion fluid does not result in an increase of the production of lactate.
Of the hydrogen ions excreted nearly 50% are excreted as ammonia.
Although the excreted amounts are small compared with the known transfer rates, they have to be taken into consideration, when studying transplacental transfers of these metabolites.

Keywords: Guinea pig placenta, metabolism, perfusion.

Zusammenfassung

Untersuchungen zum Stoffwechsel der isolierten und künstlich perfundierten Meerschweinchenplazenta
I. Ausscheidung von Wasserstoffionen, Ammoniak, Kohlendioxyd und Milchsäure und Verbrauch von Sauerstoff und Glukose

In der isolierten, perfundierten Meerschweinchenplazenta scheint Glukose ein wesentlicher Energielieferant zu sein, da Kohlendioxyd- und Laktatausscheidung durch den Glukoseabbau allein erklärbar sind.
In steady state Experimenten werden über einen Zeitraum von wenigstens 90 Minuten annähernd konstante Mengen an Wasserstoffionen, Ammoniak, Laktat und Kohlendioxyd ausgeschieden.
Die Laktatproduktion zeigt keine signifikante Abhängigkeit von der Glukosekonzentration im Perfusionsmedium.
Die Meerschweinchenplazenta scheint ihren Energiebedarf überwiegend durch anaerobe Glykolyse zu decken, da 76% der verbrauchten Glukose anaerob metabolisiert werden.
Die Plazenta produziert bei ausreichender Sauerstoffversorgung große Mengen Laktat.
Eine Verminderung des Sauerstoffpartialdruckes in der Perfusionslösung führt nicht zu einer Steigerung der Laktatproduktion. Etwa 50% der Wasserstoffionen werden als Ammoniak ausgeschieden. Obwohl die Produktionsraten im Vergleich zu den bekannten Transportraten klein sind, sollten die Stoffwechselmetaboliten bei transplazentaren Transportuntersuchungen dieser Substanzen Berücksichtigung finden.

Schlüsselwörter: Meerschweinchenplazenta, Perfusion, Stoffwechsel.

Résumé

Le métabolisme du placenta de cobaye isolé et artificiellement perfusé
I. Sécrétion des ions d'hydrogène, de l'ammoniac, du gaz carbonique et du lactate, et la consommation d'oxygène et de glucose

Dans le placenta isolé de cobaye et artificiellement perfusé le glucose semble être un important fournisseur d'énergie, parce que la sécrétion de lactate et de gaz carbonique s'explique seulement par la dégradation du glucose.
Dans les expériences en conditions inchangées les quantités d'hydrogène, d'ammoniac, de lactate et de gaz carbonique sont sécrétées dans une période d'au moins 90 minutes d'une manière constante.
La production de lactate ne montre aucune dépendance sensible de la concentration du glucose dans la solution de perfusion. Le placenta de cobaye semble satisfaire ses besoins d'énergie essentiellement par la glycolyse anaérobie parce que 76% du glucose consommé sont métabolisés par voie anaérobie.
Le placenta produit de grandes quantités de lactate en cas d'approvisionnement suffisant en oxygène.
Une diminution de la pression partielle d'oxygène dans la solution de perfusion n'entraîne pas d'accroissement de la production de lactate.
La moitié environ des ions d'hydrogène sont sécrétés sous forme d'ammoniac.
Bien que les taux de production soient faibles par comparaison aux taux connus de transfert, les métabolites doivent être prises en considération lorsqu' on étudie le transfert diaplacental de ces substances.

Mots-clés: Métabolisme du placenta, perfusion, placenta isolé du cobaye.

Acknowledgement: We wish to thank Ms. G. FRIEDE for her technical assistance.

Bibliography

[1] BATTAGLIA, F. C., G. MESCHIA: Principal substrates of fetal metabolism. Physiol. Reviews 58 (1978) 499

[2] BURD, L. J., M. D. JONES, M. A. SIMMONS, E. L. MAKOWSKI, G. MESCHIA, F. C. BATTAGLIA: Placental production and foetal utilization of lactate and pyruvate. Nature 254 (1975) 710

[3] DIJKHUISEN, G. H., H. VAN KESSEL, C. VAN ARKEL, J. J. W. LISMAN: Lactate production and enzymes in the placenta. Presentation at the international symposion on primate and non-primate placental transfer. 12–14 june, 1980 Rotterdam Personal communication

[4] HOLZMAN, I. R., J. A. LEMONS, G. MESCHIA, F. C. BATTAGLIA: Ammonia production by the pregnant uterus. Proceedings of the society for exp. biol. and med. 156 (1977) 27

[5] HOLZMAN, I. R., A. F. PHILLIPS, F. C. BATTAGLIA: Glucose metabolism, lactate, and ammonia production by the human placenta in vitro.: Pediat. Res. 13 (1979) 117

[6] HOWARD, J. M., K. E. KRANTZ: Transfer and use of glucose in the human placenta during in vitro perfusion and the associated effects of oxytocin and papaverine. Amer. J. Obstet. Gynec. 98 (4) (1967) 445

[7] KASTENDIECK, E., W. MOLL: The placental transfer of lactate and bicarbonate in the guinea pig. Pflügers Arch. ges. Physiol. 370 (1977) 165

[8] KAUFMANN, P.: Die Meerschweinchenplacenta und ihre Entwicklung. Z. Anat. Entwickl.-Gesch. 129 (1969) 83

[9] KRANTZ, K. E., BLAKEY, K. YOSHIDA, J. A. ROMITO: Demonstration of viability of perfused human term placenta. Obstet. and Gynec. 37 (1971) 183

[10] LEICHTWEISS, H. P., H. SCHRÖDER: L-Lactate and D-Lactate carriers on the fetal and the maternal side of the trophoblast in the isolated guinea pig placenta. Pflügers Arch. ges. Physiol. 390 (1981) 80

[11] LEICHTWEISS, H. P., H. SCHRÖDER, M. H. CARSTENSEN: Transfer of L-lactate in the isolated placenta. Pflügers Arch. ges. Physiol. Suppl. to Vol. 389 (1981) 189

[12] LONGO, L. D., M. D. PAPADOPOULUS, R. E. FOSTER II: Placental CO_2 transfer after fetal carbonic anhydrase inhibition. Amer. J. Physiol. 226, (1974) 703

[13] MOLL, W., H. GIRAD, G. GROS: Facilitated diffusion of latic acid in the guinea-pig placenta. Pflügers Arch. ges. Physiol. 385 (1980) 229

[14] SCHNEIDER, H., J. C. CHALLIER, J. DANCIS: Transfer and metabolism of glucose and lactate. Placenta, Suppl. 2 (1981) 129

[15] SCHRÖDER, H., H. P. LEICHTWEISS: Perfusion rates and the transfer of water across isolated guinea pig placenta. Amer. J. Physiol. 232 (6) (1977) H666

[16] SIMMONS, M. A., L. J. BURD, J. A. LEMMONS, M. D. JONES, R. L. SCHREINER, G. MESCHIA, F. C. BATTAGLIA: Placental utilization and conversion of glucose in vivo. Pediat. Res. 9 (1975) 279

[17] VILLEE, C. A.: The metabolism of human placenta in vitro. J. Biol. Chem. 205 (1953) 113

[18] YUDILEVICH, D. L., B. M. EATON, A. H. SHORT, H.-P. LEICHTWEISS: Glucose carriers at maternal and fetal sides of the trophoblast in guinea pig placenta. Amer. J. Physiol. 237 (5) (1979) 205

Received October 8, 1981. Accepted March 5, 1982.

Dr. M. H. Carstensen
Universitätsfrauenklinik Eppendorf
Martinistraße 52
D-2000 Hamburg 20