J. Perinat. Med. 10 (1982) 154

The metabolism of the isolated artificially perfused guinea pig placenta
II. Difference of excretion of hydrogen ions, ammonia, carbon dioxide and lactate into maternal and fetal veins

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

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

1 Introduction

The isolated, on the maternal and fetal side artificially perfused guinea pig placenta produces for at least 90 minutes hydrogen ions, ammonia, lactate and carbon dioxide and consumes glucose and oxygen [3]. Because the maternal and fetal metabolism are excluded, this model is appropriate for studying transport problems through the placental membranes. The guinea pig placenta is often used, because it is like the human a hemochorial placenta. There is, however, a difference between the two organs, as far as the circulations are concerned: **in the human placenta there is a multivillous and in the guinea pig placenta a countercurrent flow system** [1, 2, 5, 8, 14]. The guinea pig placenta consists of subunits with probably different tasks: there are areas for exchange functions (lobulus) and others, that are committed mainly to metabolic functions (interlobium) [7]. Because the interlobium has an abundant supply with maternal vessels, but only few fetal capillaries, it can be anticipated, that the products of the metabolism of the placenta are excreted in different quantities into the maternal and fetal veins. **This paper presents the rates of excretion of hydrogen ions, ammonia lactate and carbon dioxide into the maternal and fetal circulation.**

2 Methods

22 placentas of guinea pigs at the end of pregnancy (mean: day 59) are fully isolated and perfused on the maternal and fetal side with TC 199 (Difco). Further details of preparation and perfusion technique have been described previously [3, 9, 13]. The perfusion fluid is saturated with 95% O_2 and 5% CO_2, the pH is maintained at 7.40. The arterial pO_2 ranges from 450 to 550 mmHg, pCO_2 ranges from 22 to 30 mmHg. The perfusion is performed by means of syringe pumps (modified Perfusor®, BRAUN MELSUNGEN) with constant flow rates of 1.6, 3.2 or 6.2 ml/min. pH, pO_2 and pCO_2 are measured by an automatic blood gas analyser (Corning 175). Glucose is determined using the glucokinase method (Glucose Analyser, BECKMANN INSTR.) and lactate using the HOFFMANN LA ROCHE Lactate Analyser 640 or a standard enzymatic method (Monotest, BOEHRINGER, MANNHEIM). The concentration of ammonia is measured with a special ammonia electrode (PHILIPS Ammoniak Elektrode).

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

0300-5577/82/0010-0154$02.00

3 Results

The excretion of the metabolites, which are produced by the guinea pig placenta under steady state conditions, is larger on the maternal than on the fetal side (Tab. I).

The unpaired t-test shows a significant difference between maternal and fetal excretion for all metabolites (H^+, NH_3 and lactate: $p < .01$, CO_2: $p < .05$).

The excretion of H^+, CO_2 and lactate increases, as described in a previous paper, when the perfusion flow increases [3]. Of importance is not only the total perfusion flow (maternal plus fetal), but especially the maternal flow (Tab. II). If the total perfusion flow is 4.8 ml/min (maternal 1.6 and fetal 3.2) the excretion of H^+ amounts to .167 ± .076 on the maternal and to .127 ± .062 on the fetal side. At the same total perfusion flow but inverse ratio (maternal 3.2 and fetal 1.6) the total excretion of hydrogen ions increases and the difference between maternal and fetal rate of excretion becomes more evident (Tab. II C): the maternal rate exceeds the fetal rate 5.4 times.

At nearly the same flow ratio, but twice the total flow, the excretion increases significantly on both sides (Tab. II D). These results are similar to those for the other two metabolites. When the placenta

Tab. I. Excretion of hydrogen ions, ammonia, lactate and carbon dioxide of the artificially perfused guinea pig placenta, as measured in the maternal and fetal venous outflows. Mean and SD of 22 placentas. (N) = total no. of measurements (1–5 measurements per placenta).

	(10^{-6} mol × g^{-1} × min^{-1})		
	Maternal	Fetal	t-test
Hydrogen ions	.335 ± .163 (79)	.128 ± .097 (79)	$p < .01$
Ammonia	.195 ± .098 (47)	.128 ± .057 (47)	$p < .01$
Lactate	.528 ± .400 (49)	.303 ± .240 (49)	$p < .01$
Carbon dioxide	.367 ± .210 (45)	.284 ± .160 (42)	$p < .05$

Tab. II. Excretion of hydrogen ions, carbon dioxide and lactate at different flow rates. Mean and SD of 3 (A), 7 (C), 6 (D) and of 22 placentas, which are perfused with the standard flow rate of 3.2 ml × min^{-1} on both sides (B). (N) = total no. of measurements (1–5 measurements per placenta).

	(10^{-6} mol × g^{-1} × min^{-1})							
	Hydrogen		Carbon dioxide		Lactate			
	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	Q_m	Q_f
A	.167 ± .076 (13)	.127 ± .062	.209 ± .130 (8)	.191 ± .105	.338 ± .032 (5)	.165 ± .140	1.6	3.2
B	.335 ± .163 (79)	.128 ± .09	.367 ± .210 (45)	.284 ± .160	.528 ± .400 (49)	.303 ± .240	3.2	3.2
C	.283 ± .110 (34)	.052 ± .029	.335 ± .175 (14)	.125 ± .045	.410 ± .290 (12)	.185 ± .110	3.2	1.6
D	.511 ± .220 (15)	.082 ± .036	.386 ± .130 (6)	.168 ± .031	.892 ± .670 (6)	.482 ± .210	6.2	3.2
E	– (25)	.291 ± .120	– (17)	.317 ± .140	– (21)	.357 ± .099	0	3.2

Q_m = maternal flow rate (ml/min) Q_f = fetal flow rate (ml/min)

is only perfused on the fetal side (Tab. II E), the metabolism decreases slightly, the amounts excreted at the fetal side surpass those rates obtained at the same fetal flow during simultaneous maternal perfusion (Tab. II A).

Because of the anatomical structure, the reversal of the maternal perfusion should influence excretion rates of the metabolites. In Fig. 1 a typical experiment is demonstrated. During orthograde perfusion on the maternal and fetal side, excretion rates of H^+, CO_2 and lactate for any observation are larger on the maternal side. A reversal of the fetal perfusion flow does not change this relationship (3 placentas, Tab. III). If the maternal flow is reversed the amount of substances excreted at the maternal side is not different from the amount excreted at the fetal side (Tab. III and Fig. 1). If the perfusion is performed again in the normal direction on both sides, the rates of excretion on the maternal side return to their previous values. When the maternal perfusion is stopped, excretion increases on the fetal side. An additional removal of the muscles does not show any effects. The results of three placentas, which are perfused in the reverse direction on the maternal or on the fetal side (the opposite side being perfused orthograde) are summed up in Tab. III.

4 Discussion

Investigations of placental metabolism include the utilization of glucose and oxygen [4, 6, 11, 12] and the production of lactate [10, 12] and ammonia [6]. In a previous paper we could demonstrate for the isolated, dually perfused guinea pig placenta the consumption of glucose and oxygen and the production of H^+, NH_3, lactate and CO_2 for at least 90 minutes [3].

In all experiments the excretion rates on the maternal side exceed those of the fetal side (Tab. I).

The utilization of oxygen and glucose have been determined [3] as follows: .51 ± .11 oxygen and .35 ± .25 glucose. **The uptake of oxygen and glucose is also higher from the maternal than from the fetal vessels:** 57% of utilized oxygen and 72% of utilized glucose arise from the maternal side. These findings suggest, that the areas of metabolism are supplied preferably by the maternal perfusion. If the areas of metabolism contain more maternal lacunae than fetal capillaries, changing of the maternal perfusion should influence the placental metabolism more than alterations of the fetal perfusion. This is demonstrated in Tab. II. In spite of the fact that minor amounts of metabolites are usually excreted into the fetal circulation, the fetal excretion rate can increase when the maternal flow decreases. In the extreme experimental situation of solely fetal perfusion the placental metabolism seems to be reduced (Tab. II E). As a consequence **the fetus may receive larger rates of placental metabolites in the case of decreased maternal blood flow together with less nutrients (e.g. at placental insufficiency).**

The presented results are in accordance with the anatomical findings of KAUFMANN and DAVIDOFF [7, 8]: In the interlobium fetal capillaries are rare but many of the enzymes are found, which are necessary for metabolic functions (e.g. synthesis of proteins and steroids). The lobules show fetal capillaries and maternal lacunae but a

Tab. III. Excretion of hydrogen ions (H^+), carbon dioxide (CO_2) and lactate (La) at inverse perfusion flow. Total excretion = 100. Maternal and fetal rates as percentages of the total excretion. Mean and SD of 3 placentas. (N) = total no. of measurements (1–4 measurements per placenta).

Flow:	Orthograde on both sides			Inverse on maternal side			Inverse on fetal side		
	Maternal %		Fetal %	Maternal %		Fetal %	Maternal %		Fetal %
H^+	67.3 ± 30.6	(12)	32.7 ± 12.2	51.0 ± 17.8	(10)	49.0 ± 24.6	74.2 ± 18.6	(8)	25.8 ± 11.5
CO_2	58.7 ± 23.2	(11)	41.3 ± 18.8	47.9 ± 24.6	(6)	52.1 ± 30.8	60.2 ± 33.4	(4)	39.8 ± 7.4
La.	69.7 ± 40.8	(12)	30.3 ± 21.1	47.6 ± 26.0	(8)	52.4 ± 16.2	77.0 ± 27.4	(6)	23.0 ± 20.0

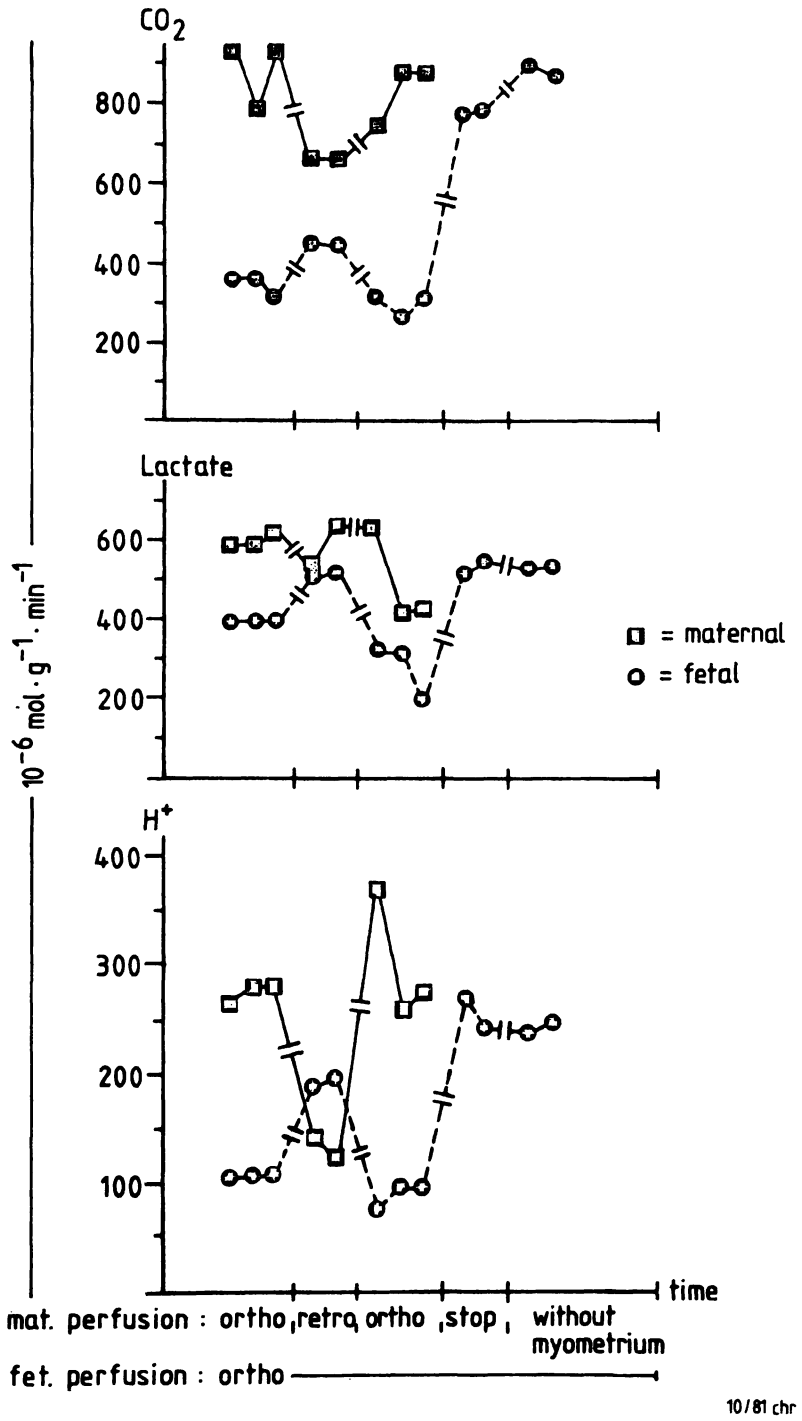

Fig. 1. Excretion rates of a dually perfused placenta. Ordinate: Excretion rates of CO_2, lactate and hydrogen (H^+) as 10^{-6} mol × g^{-1} × min^{-1}. Abscissa: time. Perfusion flow on fetal side orthograde and on maternal side orthograde, retrograde or stopped. Finally the muscles are removed.

thin syncytial tissue layer with few mitochondria between the fetal and maternal circulation. The lobules contain the labyrinth which is the proper exchange area. Fig. 2 is a diagram of anatomical and functional observations. In the direction of the maternal blood flow first this exchange area and then the metabolic area (interlobium) is perfused. Therefore the metabolites from the interlobium do not have access to the fetal circulation. This anatomical structure explains why the reversal of the maternal flow leads to an equal maternal and fetal excretion rate of metabolites:

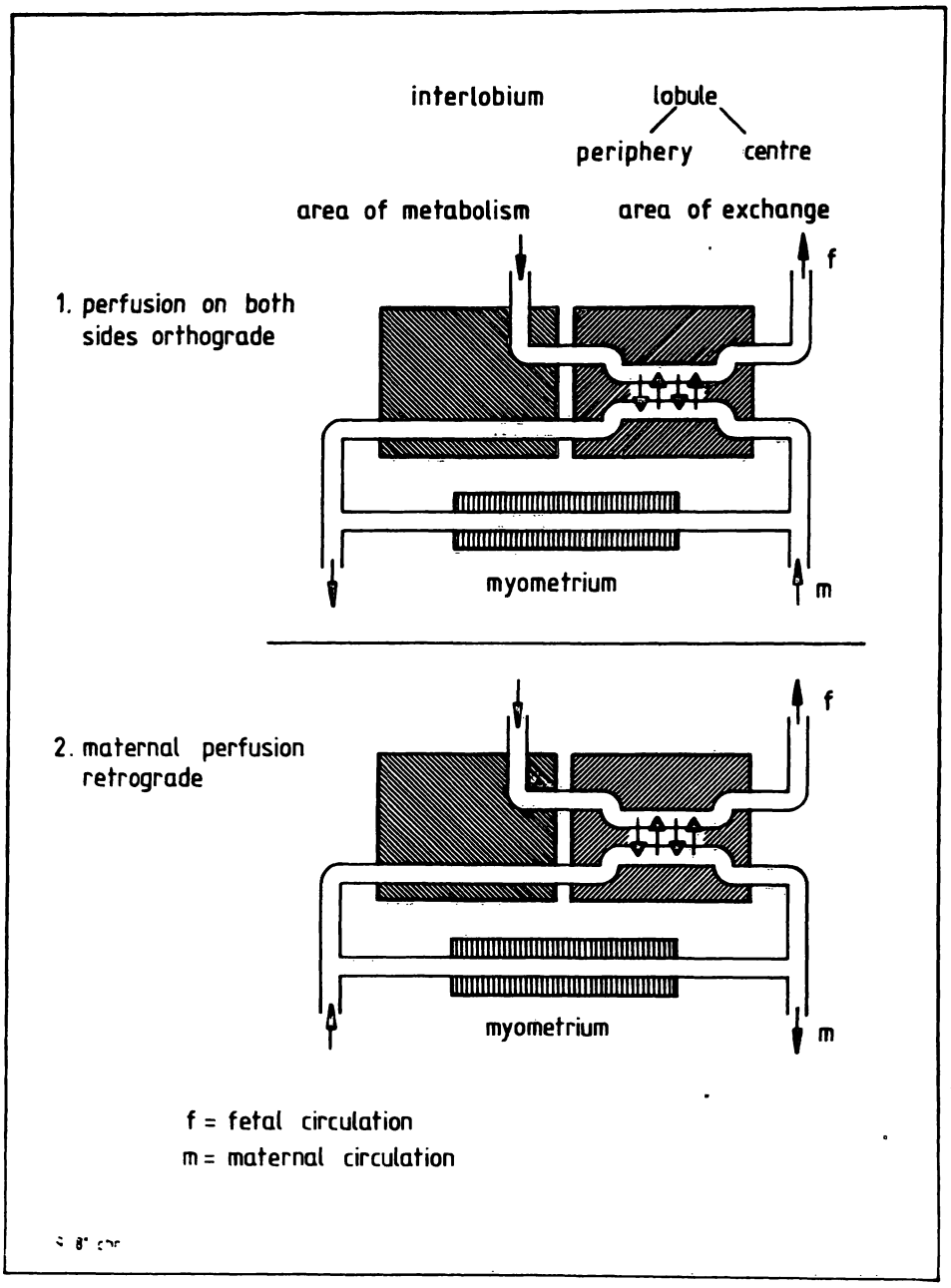

Fig. 2. Diagram of the guinea pig placenta with the subunits (lobule and interlobium), the maternal and fetal vessels, and the piece of myometrium, which is perfused in this preparation, too. The directions of the perfusion flows are indicated by arrows.

at retrograde perfusion the interlobular metabolites, which are highly diffusive substances, are rapidly exchanged with the fetal circulation. If fetal perfusion flow is reversed, the exchange is not enhanced, because the maternal metabolic area still is beyond the exchange area. Thus only the reversal of the maternal flow results in an equal distribution of metabolites to fetal and maternal veins (Tab. III).

Myometrial vessels are perfused in parallel to the maternal circulation of the placenta. If some of the metabolites are produced by the small piece of

myometrium in our preparation a difference between maternal and fetal excretion rates should remain, even when the maternal flow is reversed. **It can be concluded from the equal excretion rates at maternal flow reversal, that no measureable amounts of metabolites from the myometrium arise in our experiments.**

Morphological structure and functional results agree well. It is understandable now, that in the guinea pig placenta venous concentration differences for placental metabolites may occur. Studies of placental transfer rates of non-inert substances will have to take this into account.

Summary

The isolated and artificially perfused guinea pig placenta produces hydrogen ions, ammonia, lactate and carbon dioxide, which are secreted in significant larger amounts into the maternal than into the fetal circulation.
The excretion rates of hydrogen ions, lactate and carbon dioxide increase significantly on both sides when the perfusion flow rates are enhanced.
If the maternal perfusion flow is reversed the amount of substances excreted at the maternal side is not different from the amount excreted at the fetal side.
A reversal of the fetal perfusion flow does not influence the different excretion rates on the maternal and on the fetal side. This unequal distribution between maternal and fetal side can be explained by the anatomical structure of the placenta.
In the interlobium, which has been described as area of metabolism, fetal capillaries are rare.
Therefore the metabolites of the placenta itself arrive chiefly at the maternal circulation.

Keywords: Flow rates, isolated guinea pig placenta, metabolism.

Zusammenfassung

Untersuchungen zum Stoffwechsel der künstlich perfundierten Meerschweinchenplazenta
II. Seitendifferente Ausscheidung von Wasserstoffionen, Ammoniak, Kohlendioxyd und Laktat auf der mütterlichen und fetalen Seite

Die isolierte und künstlich perfundierte Meerschweinchenplazenta produziert Wasserstoffionen, Ammoniak, Laktat und Kohlendioxyd, wovon jeweils signifikant größere Mengen matern als fetal ausgeschieden werden.
Die Ausscheidungsraten für Wasserstoffionen, Laktat und Kohlendioxyd steigen auf beiden Seiten signifikant an, wenn die Flußraten erhöht werden.
Wird auf der maternen Seite retrograd perfundiert, unterscheiden sich die auf der maternen und der fetalen Seite ausgeschiedenen Stoffmengen nicht mehr signifikant.
Eine Umkehr des fetalen Perfusionsstromes hat dagegen keinen Einfluß auf die seitendifferenten Ausscheidungsraten.
Die seitenungleiche Ausscheidung ist mit dem Bauprinzip der Plazenta erklärbar.
Das Interlobium, welches als stoffwechselaktive Zone beschrieben wird, ist fetal kaum vaskularisiert.
Somit gelangen die Metaboliten des Plazentastoffwechsels überwiegend in die materne Zirkulation.

Schlüsselwörter: Flußraten, Meerschweinchenplacenta (isoliert), Plazentastoffwechsel.

Résumé

Le métabolisme du placenta de cobaye isolé artificiellement perfusé
II. Différence de sécrétion des ions d'hydrogène, de l'ammoniac, du gaz carbonique et du lactate dans les veines maternelles et foetales

Le placenta de cobaye isolé et artificiellement perfusé produit des ions d'hydrogene, de l'ammoniac, du lactate et du gaz carbonique, qui sont secrétés en quantités plus grandes maternellement que foetal.
Les taux de sécrétion d'hydrogene, de lactate et de gaz carbonique augmentent très nettement, lorsque les taux de d'écoulement sont élevés.
Lorsqu'on perfuse rétrogradement du côté maternel les quantités de substances sécrétées du côté maternel ne diffèrent plus de celles sécrétées du côté foetal.
Une inversion du flux foetal n'influe pas sur les taux différents des sécrétions maternelles et foetales.
La distribution inégale entre le côté maternel et foetal s'explique par la structure anatomique du placenta.
L'interlobium qui est décrit comme zone de métabolisme est peu vascularisé du côté foetal.
C'est pourquoi les métabolites du placenta lui-même parviennent surtout dans la circulation maternelle.

Mots-clés: Métabolisme du placenta, placenta isolé du cobaye, taux d'écoulement.

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

Bibliography

[1] BAILEY, D. Y.: Counter-current flow of maternal and foetal blood-stream in guinea pig placenta. J. Physiol., 242 (1974) 104P

[2] BARTELS, H., D. EL YASSIN, W. REINHARDT: Comparative studies of placental gas exchange in guinea pigs, rabbits and goats. Resp. Physiol. 2 (1967) 149

[3] CARSTENSEN, M. H., H. P. LEICHTWEISS, H. SCHRÖDER: The metabolism of the isolated and artificially perfused guinea pig placenta. I. Excretion of hydrogen ions, ammonia, carbon dioxide and lactate, and the consumption of oxygen and glucose. J. Perinat. Med. 10 (1982) 147

[4] DIAMANT, Y. Z., N. MAYOREK, S. NEUMANN, E. SHAFRIR: Enzymes of glucose and fatty acid metabolism in early and term human placenta. Amer. J. Obstet. Gynec. 121 (1975) 58

[5] DUVAL, M.: Le placenta des rougeurs. J. de l'Anat. et de Physiol. 28 (1892) 333

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

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

[8] KAUFMANN, P., M. DAVIDOFF: The guinea pig placenta. Adv. Anat. Embryol. Cell. Biol. 53 (1977) 1

[9] 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

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

[11] NESBITT, R. E. L., PH. A. RICE, J. E. ROURKE: In vitro perfusion studies of the human placenta. Gyn. Invest. 4 (1973) 243

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

[13] SCHRÖDER, H., W. STOLP, H. P. LEICHTWEISS: Measurement of Na^+-transport in the isolated, artificially perfused guinea pig placenta. Amer. J. Obstet. Gynec. 114 (1972) 51

[14] SCHRÖDER, H., H. P. LEICHTWEISS: Perfusion rates and the transfer of water across isolated guinea pig placenta. Amer. J. Physiol. 232, 6 (1977) H 666

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

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