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L-Lactate and D-Lactate Carriers on the Fetal and the Maternal Side of the Trophoblast in the Isolated Guinea Pig Placenta*

H.-P. Leichtweiß and H. Schröder

Universitätsfrauenklinik, Abteilung für experimentelle Medizin, Martinistr. 52, D-2000 Hamburg 20, Federal Republic of Germany

Abstract. The transfer of ^{14}C-labelled D- or L-lactate (test substance) relative to ^{3}H-L-glucose (control substance, extracellular marker) into the trophoblast of the isolated guinea pig placenta was determined during an open loop perfusion on both sides. Using a single passage, paired tracer dilution technique, the maximal lactate uptake (U_{max}) was derived from the venous concentration ratio of lactate to L-glucose.

A significant metabolism of L-lactate was not observed. The lactate uptake, which occurred in all placentas studied, was not significantly different on the fetal and maternal side. Within one placenta the L-lactate uptake was always higher than the D-lactate uptake. The uptake of both L- and D-lactate could be inhibited by phloretin. The lactate uptake was inversely correlated with the pH of the perfusate fluid within the range from 6.2–8. A first order saturation kinetic (Hofstee-plot) was used to approximate the relationship between the L-lactate uptake and the chemical L-lactate concentration. We conclude that similar lactate carriers exist in the membranes on both the maternal and the fetal side of the trophoblast.

Key words: Guinea pig placenta – Paired tracer dilution – Lactate carrier – Phloretin

Introduction

For the guinea pig placenta in situ a stereospecific transfer system for L-lactate has been described (Moll et al. [6, 7]). Materno-fetal and feto-maternal transfer rates are equal (Kastendieck and Moll [3]). The transfer of labelled L-lactate decreases when the concentrations of chemical L-lactate and pyruvate are elevated. A first-order kinetic is sufficient to describe the transplacental transfer system. Since transfer rates depend on pH it is concluded that the transport of L-lactate is coupled to the transfer of protons [6, 7]. There are, however, still some unresolved questions.

Even if L-lactate crosses the placental barrier more easily than D-lactate, it is possible that D-lactate may use the lactate carrier, though to a lesser extent. In this case phloretin should inhibit the transfer of D-lactate. This has not been investigated so far.

A substance which crosses the placental barrier has to pass through both the multivillous and the basal membrane of the trophoblastic layer. It is therefore of interest to study the properties of both of these membranes. This cannot be done using steady-state transfer studies, since transfer rates will always be limited by the less permeable membrane. We have used a paired tracer single injection dilution technique that has previously been applied to the isolated guinea pig placenta [2, 12]. The present study has been undertaken to demonstrate that this technique can be used to investigate the properties of both sides of the trophoblastic cells separately. It confirms the existence of a carrier for L-lactate and for D-lactate also in the isolated perfused guinea pig placenta, and it gives evidence that the fetal and maternal side of the trophoblast are not different in their ability to transfer lactate.

Methods

Placentas of guinea pigs at 55–60 days of gestation were isolated by a method described previously [5, 8, 12]. Two rings 5 cm in diameter were clamped together around the placenta to close uterine vessels. The fetal and maternal vessels were connected with a perfusion system, and the placenta and some surrounding muscular tissue were separated from the uterus and placed in a bath of Ringers solution (37°C). Fetal and meternal side were perfused with an artificial perfusion fluid consisting of TC 199 tissue culture medium (Difco) to which 1 g/l bovine albumin (Behring) and 30 g/l Dextran T 40 (Pharmacia) had been added. The fluid was gassed at 37°C with 95% O_2/5% CO_2. A pH value of 7.4 was maintained by adding $12-20\times10^{-3}$ mol $\times l^{-1}$ sodium bicarbonate. Phloretin (C. Roth, Karlsruhe, FRG) was made more soluble (up to 1 mmol/l) by addition of less than 1 g/l Pluronic F 108 (Wyando Chem. Corp.). The perfusion flow rates usually were kept constant at 3.2 ml/min on both sides by means of syringe pumps; flow rates could be changed in a stepwise manner if required by the experimental protocol. A 100 µl bolus containing about 0.2 µCi of ^{3}H-L-glucose (NEN) and about 0.2 µCi (10×10^{-9} mol $\times l^{-1}$) of ^{14}C-lactate (D or L, substances from Amersham) was injected within 1–2 s into the inflow at either the maternal or the fetal side. 30 samples each containing four drops (about 100–150 µl) were collected consecutively from the venous outflow at the injection (donor) side. After that a final sample was accumulated until the total collecting time was 6 min. Simultaneously from the acceptor side one single sample was accumulated over a 6 min period (cf. Fig. 1). During 2 h up to 8 injections (or runs) were performed. The 4-drop samples and 100 µl aliquots of the larger samples were prepared for liquid scintillation counting by addition of 10 ml Instagel (Packard). A trifold aliquot (100 µl) of the tracer mixture which had been injected was counted as well in order to relate the activities collected to the dose injected. All samples and standards were counted in a Packard Liquid Scintillation Spectrometer (3380 or 460 C) with external standardization. The standard channel ratio or spline method was used to determine the respective activities of tritium and ^{14}C.

Data from the counter were punched on paper tape and analyzed with a Dietz 621 D computer system.

Thin layer chromatography was used to discriminate between labelled L-lactate and pyruvate (personal communication of Nordmeyer, Physiologisch-Chemisches-Institut, Hamburg, FRG).

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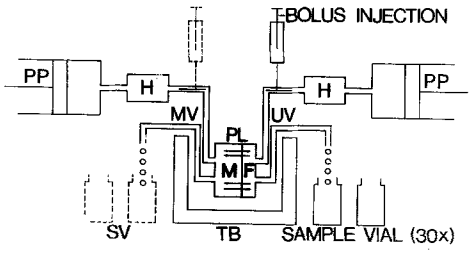

Fig. 1. Experimental set up of the dually perfused guinea pig placenta. During open loop perfusion, boli containing paired tracers are injected intraarterially, and the venous perfusate is collected in fractional samples or as one pool. *PL*, placenta; *M*, maternal blood space; *F*, fetal blood space; *MV*, mat. unterine vessels; *UV*, umbilical vessels; *PP*, piston pump; *H*, heater; *TB*, thermo. bath

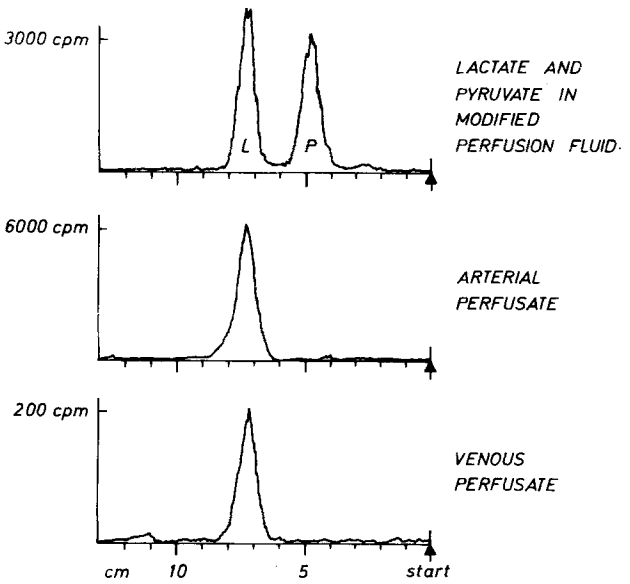

Fig. 2. Thin layer chromatography of L-lactate and pyruvate in perfusion fluid without albumin or dextran. Control run with a mixture of ^{14}C-L-lactate and ^{14}C-pyruvate on top. A bolus containing only ^{14}C-L-lactate was injected into the arterial inflow at the fetal side. The chromatography of the perfusate from the umbilical vein (bottom) shows the label only where L-lactate is to be expected

Twenty microliter samples of perfusate were dried on polygram CEL 400 layers (Macherey & Nagel). The perfusion fluid in this case did not contain albumin and dextran, since these high molecular substances falsify thin layer chromatography. After 6 h in a mixture of iso-butyric acid and ammonia complete separation was obtained. Activities were counted with a thin layer scanner (Dünnschicht-Scanner II, Berthold).

Results

In one experiment ^{14}C-L-lactate (approx. 1.5 μCi) dissolved in perfusion fluid was injected as a 0.1 ml bolus into the fetal or maternal artery. 6 samples of 25 drops each were collected at the donor side, and one sample of 2 min duration was taken from the acceptor side. Figure 2 shows the results of thin layer chromatography for a fetal venous sample when ^{14}C-L-lactate was injected into the fetal artery. Activity was only detectable where L-lactate is to be expected. The same held true for the maternal venous sample (acceptor side), and when L-lactate was injected into the maternal artery. The maximal uptake values (see below) for L-lactate in this experiment were approximately 40% on both sides. When in another experiment ^{14}C-pyruvate (Amersham) was injected instead, nearly all of the activity on the thin layer plate was detected as lactate.

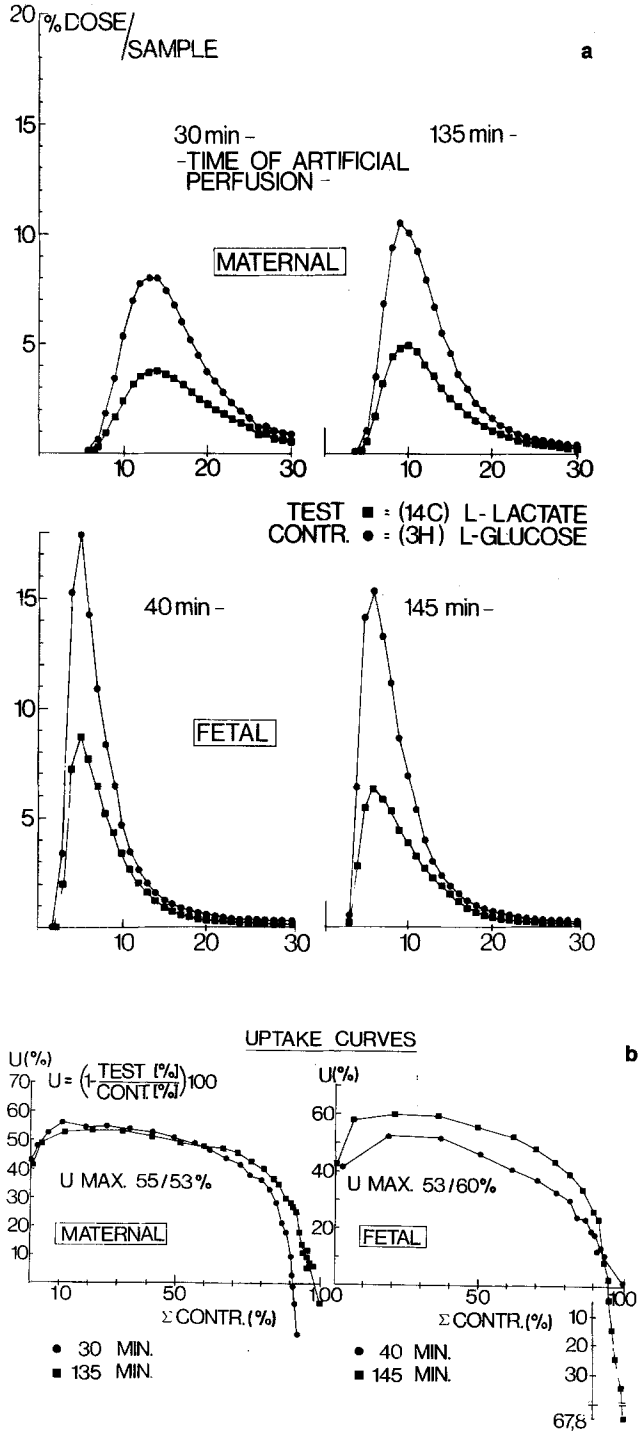

Fig. 3. (a) Typical donor side venous outflow tracer dilution curves. They are derived from sequentially collected venous samples from the injection side following the arterial injection of a bolus of ^{14}C-L-lactate and ^{3}H-L-glucose into either the maternal or the fetal inflow. *Ordinate:* activity (cpm). *Abscissa:* number of samples. **(b)** Uptake curves of the runs shown in (**a**). The uptake values calculated from each sample are plotted versus the accumulated ^{3}H-L-glucose dose of the respective sample (control). The maximal uptake (U_{max}) of ^{14}C-L-lactate is estimated by averaging 3 – 5 of the highest uptake values prior to the decline (plateau phase)

Figure 3a shows typical outflow dilution curves obtained from the donor (injection) side when the bolus containing ^{3}H-

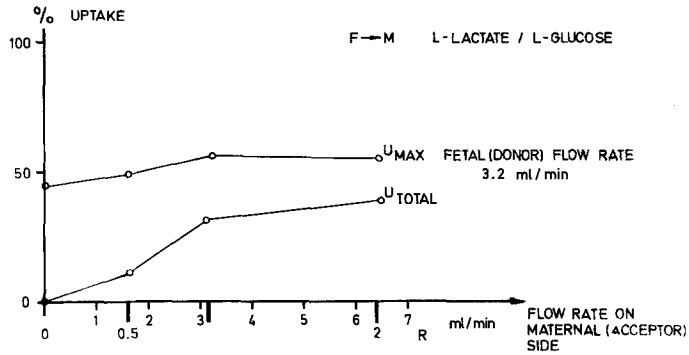

Fig. 4. Dependence of the maximal uptake (U_{max}) and the transplacental transfer (U_{tot}) for L-lactate on the flow rate of the acceptor (maternal) side. R is the ratio of the maternal to the fetal flow rate which is constant at 3.2 ml/min. *Ordinate:* uptake as percentage from injected dose. *Abscissa:* maternal flow rate (ml/min) resp. maternal to fetal flow ratio (R)

L-glucose and ^{14}C-L-lactate had been injected into either the maternal or the fetal circulation (flow rates 3.2 ml/min). The ordinate represents the relative amount of both the test (L-lactate) and control (L-glucose) tracer as percentage of the dose injected. These relative concentrations were plotted versus the number of each consecutive sample. The shorter duration of the fetal curves corresponds to the smaller volumes on the fetal side of the placenta [4]. The dilution curves if repeated under constant conditions within 145 min did not change significantly. The difference in the concentrations of the test and control tracers reflects the preferred uptake of lactate over L-glucose into the trophoblast. The percentage uptake (U) of lactate in relationship to L-glucose in each sample was calculated according to

$$U = \left[1 - \frac{\text{test (\%)}}{\text{control (\%)}}\right] \times 100 \qquad (1)$$

and was plotted for all four runs (cf. Fig. 3a) versus the accumulated control tracer recovery as percentage of dose in Fig. 3b. Thus problems were circumvented which may arise due to changes in venous outflow rates or the drop size.

The maximal uptake (U_{max}) was read from the earlier, plateau-like part of the curves. U_{max} may be regarded as being proportional to the unidirectional lactate flux from the donor blood space into the trophoblast. The decline of the uptake curves corresponds to the backflux of tracer into the vessels after the peak of the bolus has passed.

The recovery of the label from 41 placentas was nearly 100% (L-glucose: 97.5%; L-lactate: 86%).

In order to determine whether uptake values are influenced in fact mainly by those membranes that directly face the injected control and test substances, in four placentas flow rates at the acceptor side were reduced to 1.5 ml/min and zero when wash-out curves were surveyed. Typical results are represented in Fig. 4. The total uptake values (U_{tot}) were calculated from the relative amount of L-lactate and L-glucose which have disappeared from the donor side [equation (1)] during the time of bolus passage. They characterize the relative amount of substance in comparison to L-glucose actually transferred across the placental barrier. If at the acceptor side the perfusion fluid was substituted by warmed and oxygen saturated paraffin oil (paraffinum perliquidum) U_{max} values were not different from those measured with normal perfusion fluid at a flow rate of zero. No difference between the maternal and fetal sides in these experiments could be detected.

Table 1. Maximal uptake values (U_{max}) for L- and D-lactate. Data are means ± standard error of the mean from 16 placentas (L-lactate) and 7 placentas (D-lactate). Labelled lactate was injected into either the fetal or the maternal side without (control) and with Phloretin in the perfusion fluid (Phloretin). Asterisks indicate significant differences ($P < 0.01$) between adjacent groups.

	Maternal		Fetal	
	Control	Phloretin	Control	Phloretin
L-Lactate	44.5 ± 2.5*	14.3 ± 2.1	51.3 ± 3.1*	20.1 ± 1.9
D-Lactate	36 ± 3.5*	15 ± 3.7	42.1 ± 4.4*	17.8 ± 2.7

Table 2. Inhibition of ^{14}C-L-lactate uptake (U_{max}) by 1×10^{-3} mol $\times l^{-1}$ phloretin in the perfusion medium on one side of the placenta, the other side being perfused with a phloretin free medium. The paired tracer injections were made on both sides one after another. Inhibition is expressed as the percentage reduction of U_{max} from the prephloretin control. The difference of inhibition between both phloretin on the donor side and phloretin on the acceptor side is significant ($P < 0.001$).

		Phloretin on	
		Donor side	Acceptor side
Exp. 6	F	57	17
	M	82	49
Exp. 7	F	79	49
Exp. 8	F	60	25
	M	64	64
Exp. 9	F	62	24
	M	100	28
Exp. 10	M	58	33
	F	64	45
mean ± SE		69.6 ± 4.8	37.1 ± 5.1

Table 1 summarizes the results of 23 experiments, in which the maximal uptake values for L- and D-lactate in comparison to L-glucose had been measured. Flow rates were 3.2 ml/min on both sides. U_{max} for L-lactate amounts to 44–51% and for D-lactate to 36–42%. The difference between the maternal and fetal side was not significant, even when the uptake values of one placenta were compared with each other. Addition of phloretin (final concentration 1×10^{-3} mol $\times l^{-1}$) on both sides simultaneously led to a significant decrease of uptake values for L- and D-lactate. Phloretin was added solely to the donor or acceptor side in five experiments (Table 2). On the average U_{max} for L-lactate was reduced by 70% of the control value when phloretin was on the donor side. U_{max} values were only diminished by 37% of control when phloretin was dissolved in the acceptor side perfusion fluid. No attempt was made to determine the concentration of phloretin in the venous perfusate. In Table 1 U_{max} values for L- and D-lactate do not differ significantly. However, if L-lactate uptake is compared directly to D-lactate uptake within

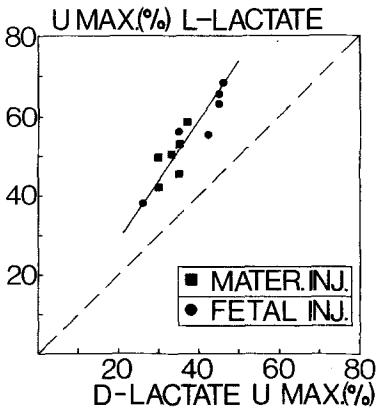

Fig. 5. Comparison between D-lactate and L-lactate maximal uptake values for 6 placentas. Both the fetal and the maternal U_{max} values of L-lactate surpass those of D-lacetate

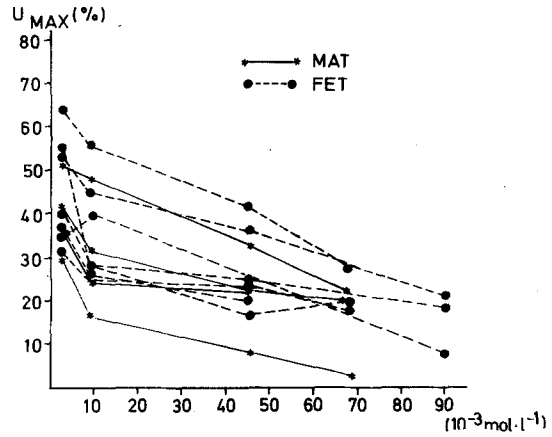

Fig. 6. Maximal uptake of labelled L-lactate and the concentration of chemical L-lactate in the perfusion fluid. *Ordinate:* maximal uptake (U_{max}). *Abscissa:* concentration of L-lactate (10^{-3} mol $\times l^{-1}$). Data are from 7 placentas (4 maternal, 7 fetal investigations). Data points for each placenta are connected (maternal side: solid line, fetal side: broken line)

one placenta, D-lactate uptake always was significantly less (Fig. 5).

In seven experiments different amounts of L-lactate (2.5, 9.0, 45.0, and 68.0 resp. 90.0×10^{-3} mol $\times l^{-1}$) had been added to the perfusion medium on both sides in order to examine a possible competitive inhibition. As Fig. 6 reveals the L-lactate uptake on both the fetal and the maternal side decreased when the lactate concentration was elevated.

pH of the perfusion fluid was changed by the addition of the appropriate amount of 1 n HCl or bicarbonate. In Fig. 7 the U_{max} is plotted versus the pH-values in the venous outflow (11 placentas). Within the range of pH between 6.2 and 8.0 the uptake decreased significantly when the pH values rose. The regression lines were not significantly different on the fetal and maternal side. The uptake of O-methyl-D-glucose was not influenced when the hydrogen ion concentration was changed.

Discussion

The trophoblastic uptake of lactate was studied in the isolated guinea pig placenta by a paired tracer dilution technique

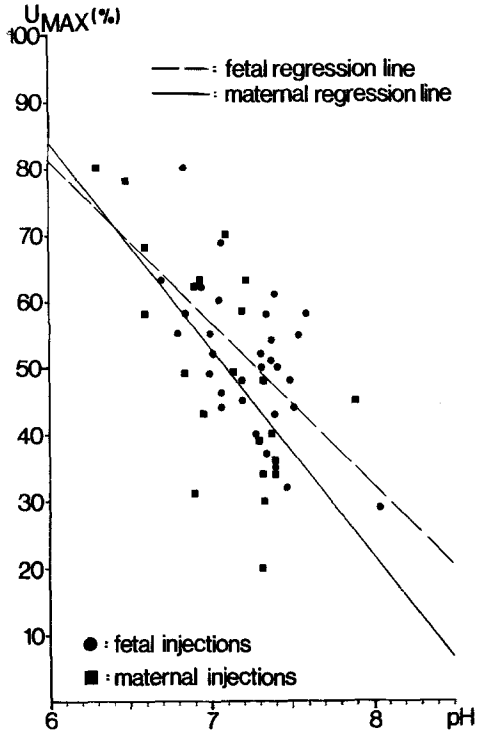

Fig. 7. Relationship between U_{max} values *(ordinate)* of L-lactate and pH values of the venous perfusate on the injection side *(abscissa)* for 11 placentas

during dually artificial open loop perfusion. One purpose was to look at the transport characteristics of the fetal and maternal surface of the trophoblastic layer from both sides. The technique has been applied sucessfully to the placenta [2, 12] as well as to other organs [10, 11].

The uptake of L-lactate into the trophoblast seems to be independent of the lactate metabolism. No other label than that of L-lactate can be detected using thin layer chromatography. Since the recovery of the label in the dual tracer experiments is close to 100% only small amounts of metabolites can remain in the placenta or are lost during handling of the samples. The conversion of pyruvate into lactate demonstrates that the placental metabolism is functioning.

L-Lactate uptake (U_{max}) into the trophoblast and L-lactate transfer (U_{tot}) are different. Whereas U_{max} is influenced only slightly by the acceptor side flow rate, U_{tot} decreases distinctly when the acceptor side flow rate is reduced. When the flow rate at the acceptor side and U_{tot} are zero, U_{max} still is close to 50%. This value is not reduced when the perfusion fluid at the acceptor side is substituted by paraffin oil, which makes the acceptor side inaccessible for hydrophilic substances. The relationship between U_{tot} and the flow ratio R is similar to that described for the diffusional transfer of water across the guinea pig placenta (Schröder and Leichtweiß [9]).

It is concluded that U_{max} represents mostly the influx characteristics of that side of the trophoblastic cells that faces the side of tracer injection. This concept is confirmed by the effects of unilateral phloretin application (Table 2), which are strongest when phloretin is present at the donor side. The single injection tracer dilution technique therefore gives the possibility to characterize the two sides of the trophoblast separately.

The uptake values for L- and D-lactate show that both substances have a space of distribution different from L-

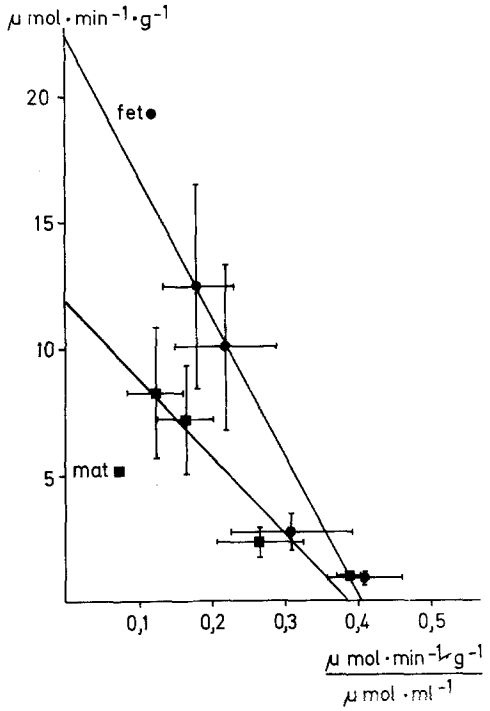

Fig. 8. Hofstee-plot for L-lactate (cf. Fig. 6) on the fetal (circles) and the maternal side (squares). Data shown are means and standard deviation (horizontal and vertical bars). Lines of symmetry, from which J_{max} and k_M are derived, are calculated from all data points

glucose which is regarded as the extracellular control marker [10]. The difference between the uptake values for L- and D-lactate, and the inhibition of the uptake by phloretin suggest that the lactate carrier described in vivo (Moll et al. [6, 7]) exists in the isolated guinea pig placenta as well.

We regard the maximal uptake values as representative for the carrier mediated unidirectional fluxes. With increasing concentration of L-lactate U_{max} and therefore the carrier flux decreases (Fig. 6). From the carrier flux and the lactate concentration, a Hofstee-plot can be constructed (Fig. 8) which allows the determination of k_M, the concentration of half-saturation, and of the maximal carrier flux J_{max}. For this case it is reasonable to calculate the carrier flux (J) as

$$J = c_{lact} \times \left[-F \times \ln\left(1 - \frac{U_{max}}{100}\right)\right] \quad (2)$$

which takes into consideration the unsteady tracer lactate concentration inside of the placenta (Cunningham et al. [1]) (c_{lact} = chemical lactate concentration in perfusion fluid, F = flow rate). For the maternal side k_M is 30, for the fetal side 49×10^{-3} mol $\times$ l^{-1}. J_{max} is on the maternal side 11 and on the fetal side 20.4×10^{-6} mol $\times$ min^{-1} $\times$ g^{-1}.

Moll et al. [7] report a J_{max} of 15×10^{-6} mol $\times$ min^{-1} $\times$ g^{-1} for the guinea pig placenta in situ which is in good agreement with our data. The k_M reported (17×10^{-3} mol $\times$ l^{-1}) is less then our value, this may be due to some impairment of the carrier function in the isolated placenta.

Because both the uptake of D-lactate and L-lactate can be impeded by phloretin in a concentration that does not alter the transfer of L-glucose (Schröder et al. [8]), we assume that a carrier mediated transport for D-lactate also exists. From our data it cannot be decided whether the L- and D-lactate system are different or not.

The uptake rate for L-lactate is higher than for D-lactate. In vivo (Moll et al. [7]) the transfer rate for L-lactate is approximately 2.5 times the value for D-lactate. If the fluxes are calculated according to equation [2] in the isolated guinea pig placenta the transfer ratio is 1.8 to 1. The difference between the uptake rates cannot be attributed to the L-lactate metabolism (see above).

The maximal uptake of L- and D-lactate are influenced by the concentration of hydrogen-ions in the perfusion fluid (Fig. 7) which again is in accordance with the findings of Moll et al. [7]. There is no significant difference in this respect between the fetal and the maternal side. The reason for this observation in our experiments is not clear. Nonionic diffusion cannot be the only cause since the pK of L-lactate is close to 3.9 and the pH of the perfusion fluid varies between 6.5 and 7.4. The general impression is that the proton concentration per se regardless of the established gradient and its direction influences the lactate uptake, though the uptake of O-methyl-D-glucose is independent of the pH value.

The U_{max} values in the guinea pig placenta seem to depend mostly on the property of membranes that separate the intracellular and extracellular space. However, it should be kept in mind that because of the vascular structure of the placenta, the possibility of flow shunts especially on the maternal side might influence the uptake parameters. This may explain why maternal uptake values are slightly, though not significantly, less than the fetal values.

Our results do not show that significant differences exist between the fetal and the maternal side of the guinea pig placenta. L-Lactate is not metabolized in significant amounts when injected into either the fetal or maternal circulation. U_{max} and thus the fluxes for L- and D-lactate are not different on the maternal and the fetal side. Inhibition of these fluxes by phloretin, the carrier kinetics and the dependence of the cellular uptake on the pH are nearly the same on both sides. We conclude that lactate carriers are situated on both the maternal and the fetal surface of the trophoblastic layer, and that they are not different.

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