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Flow-dependent transfer of antipyrine in the human placenta *in vitro*

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Summary. Placental transfer of antipyrine, a small liposoluble molecule, was investigated at varying flow rates using dual perfusion of human placental lobules. With a flow ratio equal to unity, the fœtal transport fraction (fœtal vein concentration × 100/maternal artery concentration) of antipyrine averaged 0.33. It has been suggested that effectiveness of exchange in the lobule perfused *in vitro* approximated that of a concurrent model with an antipyrine 'd' coefficient between 0.6 and 1.2, including shunting which diverted 16 p. 100 of fœtal and 13 p. 100 of maternal flow rates from the exchange area. These observations have been discussed in relation to data obtained in other mammalian placentas and in man *in vivo*.

Introduction.

The transfer of highly liposoluble and uncharged molecules across vascularized biological membranes depends mainly on arterial concentration and blood flow rate. The disposition of blood streams also intervenes when the membrane is interposed between two circulations, as in the case of the placenta. In some typical blood stream arrangements, a specific relationship exists between transfer and flow rate (Bartels and Moll, 1964 ; Meschia *et al.*, 1967). Thus, crosscurrent and countercurrent exchangers have been ascribed to sheep (Rankin and Peterson, 1969) and to guinea-pig (Moll and Kastendieck, 1977) placentas, respectively.

Although numerous studies in man have been devoted to placental circulation, the arrangement of fœtal and maternal bloodstreams *in vivo* is still not well defined. On the other hand, change in molecule transfer with variations in flow rate has received little attention. Using the relationship between the transfer of a highly diffusible molecule (antipyrine) and the flow rates of

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perfusion fluid as a point of departure, we have attempted to define the type of arrangement in the human placenta perfused *in vitro.*

The results were expressed according to parameters developed by Faber and Hart (1966) and Faber (1969) which is one of the simplest ways of describing steady-state materno-fœtal and fœto-maternal transfers in relation to flow rate. They were analyzed using the equations of certain ideal heat exchangers adapted to the placental situation.

The perfusion of the human placental lobule is suitable for this kind of study. Flow rates can be changed within desired limits and flow rates and transfer can be measured with reasonable accuracy.

The relationship between transfer and flow rate permits the effectiveness of the human placenta to be compared to that of other mammalian placentas. This study also allowed us to estimate antipyrine clearance *in vivo.*

Material and methods.

1. *Perfusion.* — The perfusion method we used has been described by Schneider, Panigel and Dancis (1972). It consists of simultaneously perfusing the two circulations of a suitable lobule in open circuit. We used Earle's buffered solution for perfusion (NaCl : 6.8 ; KCl : 0.4 ; NaH_2PO_4, H_2O : 0.125 ; $MgSO_4$, $7H_2O$: 0.2 ; $NaHCO_3$: 1.54 ; $CaCl_2$: 0.2 ; D-glucose : 1.5 g/l) equilibrated with a gas mixture containing 95 p. 100 0_2 + 5 p. 100 CO_2 or 95 p. 100 air + 5 p. 100 CO_2.

The antipyrine transfer from mother to fœtus and from fœtus to mother was measured with ^{14}C-antipyrine (NEN, 10-20 mCi/mM) or ^{3}H-antipyrine (NEN, 100-200 mCi/mM) in two separate series of experiments. Labelled antipyrine was added to the maternal or the fœtal perfusion medium depending on the study. Non-labelled antipyrine (10 mg/ml) was also added with the radioactive material.

2. *Measurement.* — Two variables were studied : antipyrine transfer and perfusion flow rate. To measure antipyrine transfer, venous perfusate samples were collected from the fœtal circuit at various intervals of perfusion time when transfer was from the maternal to the fœtal side, and from the maternal circuit when transfer was in the reverse direction.

The antipyrine in these samples, along with that in arterial perfusate samples, was separated by serial chloroform extraction. After evaporation of the chloroform phase, the residue which contained at least 93 p. 100 of the antipyrine content of the sample, was counted in diotol in a Packard scintillation spectrometer. The ratio of cpm/ml in the venous perfusate to that in the arterial perfusate gave the fraction of the substance transferred from one circuit to the other. We used fœtal transport fraction $T_F = (C_{Fv}/C_{Ma}) \times 100$ to study antipyrine transfer from the maternal to the fœtal side, and maternal transport fraction $T_M = (C_{Mv}/C_{Fa}) \times 100$ when antipyrine transfer was from the fœtal to the maternal side. T represented the transport fraction (in p. 100), C the cpm/ml of radioactive material in the perfusion medium, M and F the maternal and fœtal perfusion circuits, and a and v the artery and vein.

Flow rates were measured with Brooks R 2 15 A flowmeters at the arterial and venous ends of the fœtal circuit. A single flowmeter measured the flow rate

in the maternal arterial circuit. The flow ratio R was calculated by dividing fœtal flow rate (Q_F) by maternal flow rate (Q_M). A flow ratio equal to unity was obtained for flow rates around 10 ml/min. Low flow ratios were obtained by lowering the fœtal flow rate and high flow ratios by decreasing the maternal one. The perfusion pressure was kept under 40 mm Hg in the fœtal circuit and under 90 mm Hg in the maternal one.

3. *Data analysis.* — Transport fraction and related flow ratio were measured in 20 experiments. T_M and T_F values were grouped according to their flow ratio R into cells ranging from 0 to 7.23 and with a width of 0.15. Only maximal T_M and T_F values were retained for calculation within each flow ratio cell. This permitted us to eliminate the low transport fractions resulting from incorrect superposition of maternal and fœtal circulations.

4. *Comparison of ideal exchangers.* — We used concurrent, countercurrent and crosscurrent models. For concurrent and countercurrent exchangers, respectively, (Faber, 1969), the following equations were employed :

$$T_M = \frac{R(E-1)}{E(R+1)} \quad \text{where} \quad E = \exp\frac{d(R+1)}{R}$$

$$T_M = \frac{R(E-1)}{(R.E)-1} \quad \text{where} \quad E = \exp\frac{d(R-1)}{R}.$$

For the crosscurrent exchanger (Moll and Kastendiek, 1977), we used :

$$T_M = \frac{1}{R}\left(1 - \exp\left(-R\left(1 - \exp -\frac{d}{R}\right)\right)\right).$$

T_M and R have been defined before. d was a dimensionless coefficient obtained by dividing permeability P (number of millimoles transferred per unit of time, per unit of concentration difference, ml/min) by the product of flow rate Q (ml/min) and the ratio of the concentration of the physically dissolved solute and its total concentration in the perfusion medium. This latter ratio equalled unity for antipyrine in Earle's solution.

With the above equations we calculated d_M from T_M and R. d_F was computed from T_M and R, assuming that $T_M = R\,T_F$. A previous report (Challier *et al.*, 1977) has proved this assumption to be valid for transfer as well as for exchange in the human placental lobule perfused *in vitro*.

The effect of shunting was taken into account when using $T'_M = R\,T_F(1 - S_F)$ and $T''_M = T_M(1 - S_M)$, respectively, instead of T_M, to calculate d_F and d_M in the above equations. Two situations were considered : the finite value and the infinite value of d.

5. *Computing methods.* — The permeability coefficients d_F and d_M and the extent of shunting S_F and S_M were estimated by the non-linear least-squares method using a Gauss-Newton-type algorithm.

The fit of our experimental values to the theoretical models was evaluated by the linear regression : measured value = a + b.theoretical value. Best adjustment was considered for a = 0, b = 1 with a correlation coefficient r = 1. Three

parameters were used to estimate the fit : the value of r, the values of 'a' and 'b', and Student's t-test for 'a' and 'b'.

Results.

Antipyrine transport fractions from mother to fœtus and from fœtus to mother were measured at flow ratios ranging from 0.08 to 7.23. The results are presented in table 1.

TABLE 1
Antipyrine transfer from mother to foetus and from foetus to mother.

Cells of R.	R	Q_F	Q_M	T_F	T_M
0 – .15	0.08	0.10	12.0	67	
0.15 – .30	0.21	2.6	12.5	68	
	0.21	3.2	15.0		19
0.30 – .45	0.35	3.5	10.0	58	
0.45 – .60	0.47	5.6	12.0	51	
0.60 – .75	0.60	5.5	9.1	49	
	0.72	4.4	6.1		27
0.75 – .90	0.86	8.6	10.0	43	
	0.86	16.8	19.6		34
0.90 – 1.05	1.03	6.0	5.8	43	
1.20 – 1.35	1.34	7.8	5.8	33	
1.35 – 1.50	1.38	9.7	7.0	26	
1.50 – 1.65	1.51	11.8	7.8	22	
1.65 – 1.80	1.69	12.0	7.1	22	
2.10 – 2.25	2.22	10.0	4.5	21	
2.25 – 2.40	2.31	9.0	3.9		37
2.55 – 2.70	2.63	9.2	3.5	16.5	
2.70 – 2.85	2.73	9.6	3.5	17	
	2.81	9.0	3.2		35
2.85 – 3.00	2.88	9.8	3.4	19	
>3.00	6.48	18.8	2.9		42
>3.00	7.23	18.8	2.6		40

Maximal transport fraction in each cell of R (size : 0.15 to 3.00) is shown. R is the ratio of foetal (Q_F) and maternal (Q_M) absolute flow rates (ml/min.). T_F and T_M represent the foetal transport fraction (foetal venous/maternal arterial concentrations × 100) and the maternal transport fraction (maternal venous/foetal arterial concentrations × 100) of antipyrine respectively.

Transport fractions were plotted against flow ratios (fig. 1). At flow ratios down to 1.8, a direct relationship between T_M and R and an inverse relationship between T_F and R were observed. In the case of transport to the fœtus, a low flow ratio ($R \leqslant 0.6$) led to a nearly complete equilibration between maternal arterial and fœtal venous concentrations of antipyrine (T_F : 0.7), whereas high flow ratio (R : 1.8) brought about less complete equilibration (T_F : 0.25). The reverse occurred for the transfer of antipyrine from fœtus to mother. Above R : 1.8, the increase in flow ratio produced little or no change in transfer in either fœtal or maternal circulation.

Table 2 shows parameters a, b and r used to estimate the fit of our data to concurrent, countercurrent and crosscurrent models, permeability coefficient d

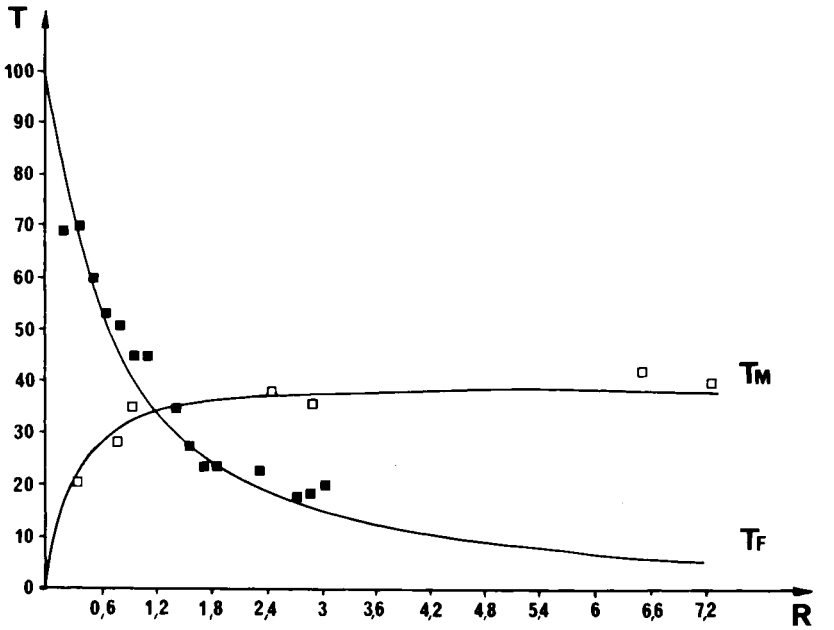

FIG. 1. — *Maternal and foetal transport fractions of antipyrine in relation to perfusion flow ratio.* T_M, T_F and R have been defined in table 1. The theoretical lines were computed with the equation of the concurrent model.

TABLE 2

Adjustment to theoretical models, permeability coefficient and shunting.

Exchangers	Transfer from mother to foetus (T_F)					Transfer from foetus to mother (T_M)				
	a	b	r	d_F	S_F	a	b	r	d_M	S_M
Countercurrent	0.024* ± 0.017	§0.941* ± 0.040	0.988	1.13	0.28	0.226* ± 0.088	1.65* ± 0.264	0.953	0.36	−0.26
Crosscurrent	0.014** ± 0.018	1.029*** ± 0.045	0.988	1.35	0.25	0.140* ± 0.032	0.608* ± 0.095	0.944	0.53	0.57
Concurrent	0.009 ± 0.022	1.019 ± 0.054	0.982	1.25	0.16	0.0003 ± 0.0049	1.002 ± 0.148	0.959	0.65	0.13
	0.076* ± 0.026	1.174* ± 0.063	0.982	∞	0.21	0.144* ± 0.031	0.600* ± 0.092	0.959	∞	0.49

§ Mean ± standard deviation.

a and b are coefficients used to evaluated the fit of our experimental values to the theoretical models. Best adjustment was considered for r = 1, a = 0, b = 1. As r was up to 0.90 in each model, a and b were tested against 0 and 1, respectively, using Student's t-test (* : $p < 0.001$, ** : $p < 0.01$, *** : $p < 0.05$).

T : transport fraction (see table 1) ; d : coefficient of permeability (permeability/flow rate) ; S : shunting (for calculation, see Material and methods) ; F and M : foetal and maternal sides, respectively.

and extent of shunting S. Significant correlation coefficients were obtained in all models. In contrast, there was a striking difference between values a and b which were found closest to their theoretical value (a = 0 and b = 1) in the concurrent model. Furthermore, it was the only model in which no significant difference was observed between measured and theoretical values of a and b at the 5 p. 100 level of significance. Thus, the concurrent model seemed to be more appropriate for describing change in transfer related to modification of flow rates in our experiments.

The d coefficients obtained from T_F and T_M in this model were $d_F = 1.25$ and $d_M = 0.65$. The difference between d_F and d_M represented a 30 p. 100 difference between RT_F and T_M. The d_F and d_M values were nearly identical when $T_M = 0.7\ RT_F$. This might reflect variability among the perfused placentas since one series of placentas was used for measuring T_M and another series for T_F. Because of this variability, the permeability coefficient of antipyrine must be considered to lie somewhere between 0.6 and 1.2.

The extent of shunting in the concurrent model represented 16 p. 100 in the fœtal circulation and 13 p. 100 in the maternal one. The hypothesis of infinite antipyrine permeability was also investigated. In that case, maternal shunting rose to 49 p. 100. However, the significant difference ($P < 0.001$) observed in comparing measured and theoretical values of a and b tended to disprove this hypothesis.

Discussion.

This study confirms previous findings on the flow dependence of antipyrine placental transfer from mother to fœtus (Schneider *et al.,* 1972). Further, it shows flow-dependent transfer of antipyrine from fœtus to mother which was of smaller magnitude than that seen from mother to fœtus.

Most mammalian placentas exhibit such flow dependence for highly liposoluble compounds. However, depending on the efficiency of the placental exchanger, great differences in materno-fœtal transport fractions are observed at similar flow ratio. Transport fractions of around 80 p. 100 have been found for acetylene in rabbit (Faber and Hart, 1967) and for nitrous oxide in guinea-pig (Moll and Kastendiek, 1977) placentas for flow ratios near unity (fig. 2). The transport fractions reported for antipyrine in sheep (Meschia *et al.,* 1966) were between 35 and 45 p. 100 at the same flow ratio. A value of 30 p. 100 was found in goat (Rankin and Peterson, 1969). An antipyrine transport fraction of around 33 p. 100 was noted in the present study on the human placenta perfused *in vitro.* This figure agrees well with that reported by Faber and Hart (1966) for various investigations on oxygen transport in man. Moreover, it is of the same order as that observed for antipyrine in rhesus monkey by Behrman *et al.* (1969) (see Faber, 1977).

The most efficient mammalian placentas (rabbit and guinea-pig) have been thought to be countercurrent exchanger types and the less efficient ones (goat and sheep) crosscurrent exchangers. The human placenta has been considered primarily a pool exchanger (Prystowski, 1957). According to Bartels and Moll

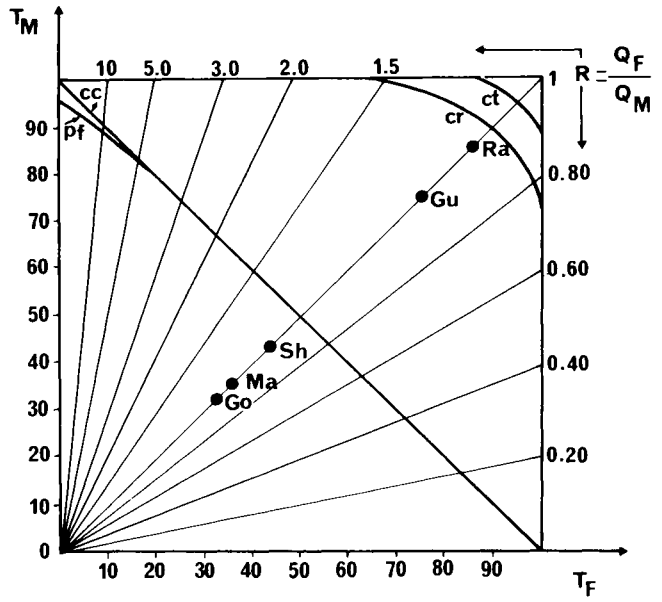

FIG. 2. — *Efficiency of exchange in mammalian placentas.* The theoretical T-R lines corresponding to nearly maximal permeability (d = 30) are presented for countercurrent (ct), crosscurrent (cr), concurrent (cc), pool flow (pf) exchangers. (●) transport fraction for R = 1 in rabbit (Ra), guinea pig (Gu), sheep (sh), man (Ma) present study, goat (Go).

(1964) and Metcalfe *et al.* (1964), the vascular arrangement in humans has the character of a multivillous streambed and the exchange pattern that of a crosscurrent system. In contrast, the relationship between antipyrine transfer and the flow ratios observed in this study indicates that the human placenta behaves as the less efficient exchangers, i.e. concurrent, pool or double pool flow. It was not possible to determine which of these exchangers was involved because their pattern of exchange was very close for d values as low as 0.6 and 1.2. The concurrent model was chosen arbitrarily for calculation, and it was found to fit our data well. This observation however does not implicate a concurrent arrangement *in vivo* where hemodynamic studies (Ramsey, 1968) suggest the presence of a mixed exchanger. Nevertheless, this model appears more appropriate than the crosscurrent or the countercurrent model for describing the change in transfer of flow-limited molecules with variations in flow rate *in vitro.*

These findings imply that the human placenta functions as a vein-to-vein rather than an artery-to-artery equilibrator. The umbilical and utero-placental venous concentrations of antipyrine nearly equilibrate at flow ratio close to unity. This is in contrast to guinea-pig and rabbit placentas in which equilibration of umbilical venous and utero-placental arterial concentrations of highly diffusible molecules has been reported at similar flow ratio.

Non-exchanging shunt might be responsible for the low transfer efficiency of the human placenta *in vitro.* Our data suggest that about 10 % of the perfu-

sion medium is diverted from the area of exchange in the foetal as well as in the maternal circulation. This figure is lower than those reported in sheep by Rankin and Peterson (1969) who found 23 % for foetal shunt and 36 % for maternal shunt using carbon monoxide. The extent of shunting in rhesus monkey has been estimated at about 35 % in maternal circulation and about 30 % in foetal circulation (Faber, 1977). Schneider *et al.* (1972) estimated maternal shunt of 50 % on the basis of infinite antipyrine permeability. Our results suggest that this hypothesis is unacceptable. Thus, it is likely that the low efficiency of human placenta should be ascribed to the exchanger rather than to shunting. Other factors as uneven distribution of perfusion flow rates or permeability could be implicated (Faber, 1969). Their extent has not been investigated in the present study.

The existence of such shunting has not been clearly demonstrated so far in human placenta. Mayer *et al.* (1956) have suggested that the paravascular network constitutes the anatomical shunt in foetal circulation. This is consistent with the absence of large-diameter shunts in villous circulation reported by Penfold *et al.* (1981). In maternal circulation there is no anatomical support for these shunts. It is likely that shunting takes place by short-circuiting of blood flow between utero-placental artery and veins (by-passing the villi).

An interesting aspect of this study is the estimation of placental antipyrine clearance *in vivo*. The value of uterine blood flow rate reported by Metcalfe *et al.* (1955) averages 492 ml/min for a pregnant uterus weighing 5 kg. Umbilical blood flow rate averages 248 ml/min (Stembera *et al.*, 1965) for a foetus of 3.3 kg. The foeto-maternal flow ratio expected from these values is 0.50. At this ratio, transport fractions of 55 % from mother to foetus and of 27 % from foetus to mother were obtained on our T-R plot. In that case, antipyrine clearance would be approximately 135 ml/min *in vivo.*

It has been previously shown that antipyrine and water clearances are not significantly different in perfused human placenta (Challier *et al.*, 1977) or in sheep cotyledon (Meschia *et al.*, 1967). The figure of antipyrine clearance estimated in the present study is about two times that observed by Hutchinson *et al.* (1959) for water, using compartmental analysis. Among the reasons which might explain this discrepancy are a probable underestimation of the flow ratio due to non-placental uterine blood flow, or enhanced permeability *in vitro* of the trophoblastic membrane due to water-filled channels (Hedley and Bradbury, 1980).

Conclusion.

This study shows a striking difference in hemochorial placentas as regards their exchange efficiency. This difference seems related to a marked species variation in the arrangement of the vascular beds.

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Résumé. *Relation entre le transfert d'antipyrine et le débit circulatoire dans le placenta humain perfusé in vitro.*

Le transfert placentaire de l'antipyrine, une petite molécule très liposoluble, a été étudié à différents débits circulatoires au cours de la double perfusion de lobules placentaires humains. La fraction de transfert de l'antipyrine (concentration dans la veine fœtale × 100/concentration dans l'artère maternelle) au rapport de débit égal à l'unité est de 33 %. L'efficacité d'échange du lobule perfusé *in vitro* est comparable à celle d'un modèle d'échange concourant présentant des shunts qui détournent 16 % du débit circulatoire fœtal et 13 % du débit circulatoire maternel de la zone d'échange. Le coefficient de perméabilité « d » de l'antipyrine dans ce modèle est compris entre 0.6 et 1.2. Ces observations sont discutées en fonction des résultats obtenus dans d'autres placentas de mammifères et dans l'espèce humaine *in vivo*.

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