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Transfer of Heparin Across the Human Perfused Placental Lobule

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Abstract—A system of dual perfusion of an isolated lobule of term human placenta was used as a model to study the transfer of heparin from maternal to foetal circulation. The metabolic viability of the system was assessed by measuring β-HCG and alkaline phosphatase levels in both maternal and foetal perfusates. Creatinine and antipyrine were used as markers to determine juxtaposition of the maternal and foetal circulations. Results of this study indicate that following administration of a single bolus dose of heparin into the maternal circulation, its concentration declined slowly from 99·01 ± 2·98 at 15 min to 97·23 ± 4·12% and transfer of heparin in the foetal circulation was linear and increased from 0·10% ± 0·05% at 15 min to 0·46 ± 0·19% over a period of 120 min. The maternal (MAUC) and foetal (FAUC) concentration-time integrals were found to be 70160 ± 1332 and 340 ± 30 int. units min mL^{-1}, respectively. Placental permeability of heparin and creatinine, calculated as the ratio of foetal concentration to the integral maternal-foetal concentration difference, was $8{\cdot}65 \times 10^{-5} \pm 0{\cdot}80 \times 10^{-5}$ and 0·033 ± 0·006 mL min^{-1} g^{-1} of perfused placental weight, respectively. These data suggest that heparin was transferred from the maternal to the foetal circulation in small quantities.

Thrombo-embolism occurs in approximately 1·2% of all pregnancies, a sixfold higher incidence compared with that of the non-pregnant state (Royal College of General Practitioners 1967; Hellgren & Blomback 1981). Thrombo-embolic disorders constitute the single most common cause of maternal mortality in England and Wales in that they account for 20% of maternal deaths in the UK (DHSS 1991). Similar statistics are also obtained from US studies (Atrash et al 1990). Furthermore, these disorders account for significant morbidity in survivors due to symptomatic pulmonary hypertension and its sequelae (Sharma et al 1980).

One of the factors which contributes markedly to the high mortality figures associated with thrombo-embolic disease during pregnancy is the restricted use of anticoagulant drugs for both prophylaxis and treatment of deep venous thrombosis (DVT). It is known that treatment with anticoagulant drugs constitutes a lifeline for patients with DVT and pulmonary embolism (PE). For instance Villasanta (1965), in an uncontrolled study, showed that untreated DVT is associated with a 15–24% incidence of pulmonary embolism with a mortality rate of approximately 12–15%. Treatment with anticoagulant drugs could reduce the incidence of pulmonary embolus associated with DVT to 4·5% with a mortality rate of 0·7% (Rutherford & Phelan 1986; Sipes & Weiner 1990). Many other studies also substantiate the value of anticoagulant drugs in reducing maternal mortality and morbidity in PE (Hirsh et al 1972; Bonnar 1981; Painter 1983). Clearly these studies indicate that the best treatment for pulmonary embolus is early treatment of DVT with anticoagulant drugs such as warfarin and heparin. However, use of these drugs in pregnancy is severely limited due to their foetal or maternal side-effects. For instance, use of the oral anticoagulant drug warfarin is somewhat restricted during pregnancy because of its tetratogenic effects which lead to neurological abnormalities in the foetus (Pettifor & Benson 1975; Stevenson et al 1980; Kaplan et al 1982; Greer 1989). Therefore parenteral administration of heparin has been used widely for prophylaxis as well as for treatment of DVT/PE throughout the period of gestation. Some reports suggest that long term use of heparin can be associated with foetal morbidity and mortality due to prematurity and still-birth (Hall et al 1980; Majerus et al 1990). The precise mechanism by which heparin causes foeticidal effects is poorly understood as heparin is said not to cross the placenta (Flessa et al 1965). However, there is no direct evidence on transplacental transfer of heparin in man, and foetal effects of heparin have not been ascertained by correlation with the degree of maternal-to-foetal transfer of these drugs. This study was undertaken to characterize the uptake and transport of heparin across the isolated lobule of perfused term human placenta.

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Materials and Methods

Materials

Sodium heparin (mol. wt 15000 Da) was obtained from Paines and Byrne Ltd., Greenford, UK. Azure A dye, dextran and sodium citrate were obtained from Sigma Chemical Co., Poole, Dorset. TC-199 medium was obtained from Pharmacia LKB Biotechnology, Sweden.

Perfusion technique

The technique for perfusing isolated human placenta has remained essentially unchanged since initially described by Contractor & Stannard (1983). Briefly, placentas were obtained from normal term vaginal deliveries or elective caeserean section deliveries and transported within minutes to the perfusion laboratory. A pre-heparinized cannula was threaded into the umbilical vein up to the point of insertion of the cord on the chorionic plate. The autologous foetal

blood from the whole placenta was allowed to drain through the cannula into the reservoir to be mixed with the TC-199 perfusion fluid.

A non-traumatized placental lobule was selected which had its arterial supply from a single accessible foetal chorionic artery with little or no visible anastomotic supply from neighbouring vessels, and had its venous drainage via a single good-sized vein. Placental perfusion of the foetal capillary network in a selected placental lobule was established within 5 min of obtaining the placenta, to minimize the period of tissue hypoxia and ischaemia by cannulating the chorionic artery. The foetal venous catheter was guided into the main chorionic vein which drained the perfused area. The cannula was then threaded into the lobular vein as far as feasible. This manoeuvre brought the arterial and the venous cannulae in close proximity, and consequently ensured proper venous outflow. The venous catheter was ligated securely in place to avoid slipping off of the cannula during the experimental period. It was then connected to the foetal perfusion circuit. The foetal vasculature was perfused at a pressure of 60–80 mm Hg and with a flow rate of 6–8 mL min^{-1}. The circuit volume was about 110–120 mL with a haematocrit of 12–18%.

The placenta was turned over with the maternal surface facing upwards. The area of placenta being perfused from the foetal side was identified by the presence of slight blanching which resulted from low foetal haematocrit. The intervillous space of the lobule was cannulated by inserting five polyvinyl maternal arterial catheters to a depth of 0·5–1 cm through its basal plate. Each cannula was equally spaced in the perfused lobule. The venous effluent was drained from the surface of the lobule by gravity into the reservoir to complete the closed circuit. The drainage was further enhanced by placing the dish on the top of an eccentric rocking stand which also prevented pooling and stagnation of the maternal perfusate. The perfused lobule was isolated from the rest of the placenta by a perspex chamber. The maternal perfusate was a mixture of autologous maternal blood drained from the intervillous space and Tc-199 and was oxygenated with 5% CO_2–95% O_2. The maternal circulating volume was 150–160 mL with a haematocrit of 5–10%. The perfusion pressure was 18–25 mm Hg with an outflow rate of 20–24 mL min^{-1}.

Following establishment of the dual circulation in the placenta, oxygenation of the foetal perfusate was discontinued and only the maternal perfusate was oxygenated to simulate normal physiological conditions. The pH, pO_2, and pCO_2 of both perfusates were monitored at frequent intervals throughout the experimental period using a Corning 165 blood gas analyser (Corning instruments). Parameters were kept within normal physiological ranges in both circulations by altering the gas mixtures. The pH was controlled by the addition of bicarbonate when necessary.

The placental preparation was observed for 5–10 min to ensure that all the physiological conditions of the experimental conditions were met before undertaking the transfer kinetics study. During the period of observation, if foetal perfusion pressure or venous outflow were not within physiological ranges or if the foetal circulating volume dropped, due to a hydrostatic fluid shift with the maternal circuit, the placental preparation was abandoned. In all experiments, transplacental transfer of the slowly diffusing molecule creatinine was used as a marker substance to monitor the normal functioning of the perfusion system. Only data obtained from those experiments in which the creatinine transfer fell within the pre-defined range of 8–16% of the administered dose at 2 h, were subsequently analysed (Eaton et al 1985).

Experimental design

Two millilitres of blood was removed from both the maternal and the foetal circulation before addition to the test substance, as control for drug and endogenous creatinine levels. A solution containing 100 000 int. units heparin, and 30 mg of creatinine was injected into the maternal arterial distribution head over 6 min (the time required for one complete circulation). In four experiments, 30 mg of antipyrine was also added to the maternal circulation in order to measure its placental clearance. In these experiments, both maternal and foetal compartments were not recirculated. Two millilitres of perfusate was sampled at 15 min intervals from the foetal circulation and at 30 min intervals from the maternal circulation throughout the 2 h perfusion period. The volumes of the samples withdrawn from the maternal and foetal circulations were replaced by injecting equal volumes of fresh maternal or foetal perfusates into the corresponding circulations. Samples were centrifuged at 3000 *g* for 15 min to sediment red cells, and the supernatant was assayed for heparin and creatinine.

At the end of the perfusion period, both circuits were completely drained and their volumes were measured. The

Table 1. Concentration of heparin in foetal circulation in experiments with citrate-based perfusate.

	Time (min)							
Exp. no.	15	30	45	60	75	90	105	120
10*	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
11	0·13	0·21	0·31	0·43	0·53	0·61	0·68	0·71
12	0·09	0·14	0·14	0·16	0·18	0·24	0·29	0·30
14	0·11	0·11	0·16	0·27	0·33	0·36	0·48	0·48
16	0·11	0·17	0·28	0·36	0·38	0·50	0·57	0·60
17	0·17	0·28	0·38	0·42	0·49	0·57	0·60	0·63
Mean	0·10	0·15	0·21	0·27	0·32	0·38	0·44	0·45
s.d.	0·05	0·09	0·14	0·17	0·20	0·23	0·25	0·26

* In this experiment, concentration of heparin was undetectable as 5000 int. units L^{-1} was injected in the maternal circuit as opposed to 100 000 int. units mL^{-1} in other experiments. Sensitivity of the heparin assay was 0·01 units L^{-1}. All values are expressed as percentage of initial dose added to the maternal circulation at 0 time.

Table 2. Foetal concentration of heparin in experiments with heparin-based perfusate.

	Time (min)							
Exp. no.	15	30	45	60	75	90	105	120
19	0·03	0·11	0·21	0·28	0·29	0·32	0·37	0·52
20	0·08	0·14	0·16	0·22	0·28	0·35	0·39	0·45
21	0·05	0·08	0·11	0·15	0·18	0·22	0·26	0·29
22	0·11	0·17	0·29	0·36	0·41	0·48	0·52	0·54
23	0·10	0·18	0·22	0·28	0·31	0·36	0·40	0·42
24	0·19	0·26	0·32	0·43	0·49	0·55	0·58	0·62
Mean	0·09	0·16	0·22	0·29	0·33	0·38	0·42	0·47
s.d.	0·06	0·06	0·08	0·10	0·11	0·12	0·11	0·11

See legend to Table 1 for details.

remaining concentrations of heparin and creatinine in the maternal circuit and that transferred to the foetal circuit were determined by multiplying the circuit volume by the concentration in 1 mL of perfusate at each sampling point.

The perfused placental lobule within the chamber was excised at the end of the experiment, and pressure blotted to remove the perfusate from the intervillous space and weighed. The placental tissue was homogenized in 2–3 vol of water using an Ultra-Turrax high speed homogenizer (Janke and Kunkel, KG., Staufen, Germany). The final volume of the homogenate was measured. Ten millilitres of the homogenate was centrifuged, and concentration of heparin in the supernatant was determined.

The concentration of drug in the maternal and foetal circulation was expressed as a percentage of the dose added after it was corrected for background level, the circuit volume, and the amount removed from the previous samples. The concentration in the perfused placental tissue was expressed as percentage of the initial dose of drug added to the maternal circulation after correction was made for the background level, which was obtained from the non-perfused placental tissue.

Placental permeability of heparin and creatinine were calculated by using the following equation (Thornburg et al 1988):

$$PS = N/(\text{wt placenta} \times [C_m - C_f]dt\ \text{mL} \times \text{min}^{-1} \times g^{-1}) \quad (1)$$

PS = placental permeability in mL min^{-1} g^{-1} of perfused placental tissue, N = total foetal plasma concentration at 120 min, $[C_m - C_f]dt$ = integrated concentration difference across the placenta between 0 and 120 min of the experiment. This was calculated by subtracting total area under the curve for the maternal circulation (MAUC) from the area under the curve for the foetal circulation (FAUC).

The coefficients of free diffusion in water of heparin and creatinine at 37°C were calculated from their mol. wts using the following formula (Faber 1973):

$$D = 5{\cdot}1 \times 10^{-5}/(\text{mol. wt}^{0{\cdot}40}) \times 0{\cdot}649 \quad (2)$$

Molecular size of heparin and creatinine were calculated by using the Einstein-Stokes formula. Clearance of antipyrine and clearance index of heparin were calculated by using the formula given by Liebes et al (1990).

Heparin

The concentration of heparin in the perfusate sample was determined by the colorimetric method of Klein et al (1982). The assay is based on the reaction of heparin with azure dye. Heparin forms a complex with azure dye to produce a blue colour with an absorbance maximum at 620 nm on a Zeiss PMQ II spectrophotometer. The concentration of heparin in the samples was inversely proportional to the intensity of the colour produced. A standard curve of heparin was constructed over a concentration range of 0·1–10 int. units mL^{-1} (r = 0·99).

Creatinine

The concentration of creatinine was determined by the colorimetric assay of Heinegard & Tiderstrom (1973) using a commercial kit (Sigma) on a Zeiss PMQ II spectrophotometer at a wavelength of 500 nm. A standard curve was prepared to establish the linearity limit between the absorbance and the concentration of creatinine tested, i.e. at a concentration of 1 and 10 mg dL^{-1} (r = 0·99).

Antipyrine

The concentration of antipyrine was measured by the spectrophotometric assay of Brodie et al (1949).

β-HCG

Both maternal and foetal samples were assayed for β-HCG by radioimmunoassay (Kardana & Bagshawe 1976).

Alkaline phosphatase

Concentrations of alkaline phosphate in the maternal and foetal perfusate were measured by a monoclonal antibody based solid-phase enzyme binding assay (Contractor et al 1985).

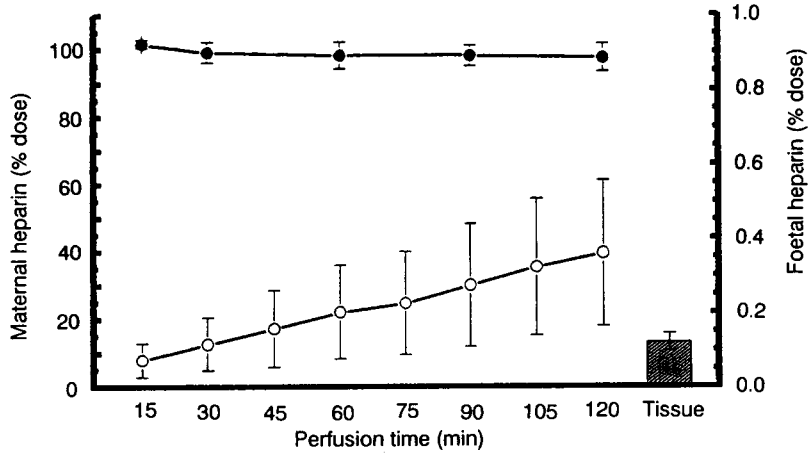

FIG. 1. Distribution of heparin in maternal (●) and foetal (○) circulations and in the perfused placental tissue (■) during 2 h of perfusion. Data obtained from 12 perfusion experiments were plotted as a percentage of the initial dose of heparin administered in the maternal circuit. All values were expressed as mean ± s.d. (n = 12).

Table 3. Heparin permeability measurements on eleven placentae.

Exp. no.	Foetal concn (int. units mL^{-1})	MAUC (int. units min mL^{-1})	FAUC (int. units min mL^{-1})	Wt of placenta (g)	PS $\times 10^{-5}$ (mL min^{-1} g^{-1})
11	708·82	65824·98	411·38	83·00	13·0
12	291·71	63897·08	254·25	78·60	5·80
14	478·50	74700·76	312·75	96·20	6·60
16	545·16	70215·87	388·50	91·80	8·50
17	631·04	75407·56	480·75	74·50	11·3
19	522·83	70178·06	274·88	76·40	9·70
20	445·28	66730·67	332·25	81·03	8·22
21	257·92	77479·94	137·25	87·50	3·80
22	539·58	66476·57	419·65	78·20	10·4
23	419·94	72605·33	300·49	80·60	7·20
24	619·99	68209·37	427·00	86·40	10·5
Mean	496·43	70156·92	339·92	83·11	8·65
s.d.	131·78	4208·97	93·53	6·40	2·50
s.e.m.	41·70	1331·95	29·60	2·03	0·80

PS = placental permeability.

Statistics

All values are expressed as mean ± s.e.m. unless stated otherwise. Student's *t*-test was used to compare data between the two groups. $P < 0{\cdot}05$ was considered statistically significant. Area under the curve (AUC) was calculated by the trapezoidal rule. If the concentration of drugs at 15, 30, 45, 60, 75, 90, 105 and 120 min are a1, a2, a3, a4, a5, a6, a7, and a8, AUC in the foetal circulation (FAUC) was calculated by using the following formula $(a1 + 2a2 + 2a3 + 2a4 + 2a5 + 2a6 + 2a7 + a8) \times 7{\cdot}5$, and that in the maternal circulation (MAUC) by $(a1 + 3a2 + 4a4 + 4a6 + 2a8) \times 7{\cdot}5$.

Results

Twelve placental preparations were used to ascertain the transfer kinetics of heparin from the maternal to the foetal circulation. In 6 experimetns (nos 10, 11, 12, 14, 16 and 17), 3·8% sodium citrate was used as an anticoagulant for both maternal and foetal perfusates while in another six experiments (nos 19, 20, 21, 22, 23 and 24), heparin was used as an anticoagulant for both perfusates. In the latter experiments, the transfer of heparin across the placenta was measured above the basal level of heparin ($4{\cdot}1 \pm 0{\cdot}02$ int. units mL^{-1}). The basal concentration of heparin was subtracted from all values before any calculation was undertaken.

Foetal concentrations of heparin in both groups are shown in Tables 1 and 2. Since there was no statistical difference in the concentration of heparin between the placental preparations of these two groups, the transfer kinetics data of heparin of these groups were pooled and the amount transferred into the foetal circulation was expressed as a percentage of the added dose, and plotted against the perfusion time.

Fig. 1. shows that the concentration of heparin in the foetal circulation rose steadily in a linear fashion from $0{\cdot}10 \pm 0{\cdot}05\%$ at 15 min to $0{\cdot}46 \pm 0{\cdot}19\%$ at 120 min ($r = 0{\cdot}999$; $n = 12$), the maximum concentration being attained at 120 min ($0{\cdot}46 \pm 0{\cdot}19\%$). In the maternal circulation, very little change in the heparin concentration occurred during the experimental period of 2 h ($99{\cdot}01 \pm 2{\cdot}98\%$ at 15 min compared with $98{\cdot}89 \pm 3{\cdot}89\%$ at 120 min). A line weighted for the standard deviations of each mean has been fitted for the foetal concentration of heparin between 15 and 120 min. From the slope of this line the transfer rate of heparin was calculated as 0·28% of total dose h^{-1} i.e. $0{\cdot}039 \pm 0{\cdot}0045$ int. units min^{-1} mL^{-1}. Perfused placental tissue uptake of heparin was $0{\cdot}12 \pm 0{\cdot}2\%$ of initial dose added. Recovery of heparin in these experiments was $98{\cdot}23 \pm 1{\cdot}56\%$. All the above values are expressed as mean ± s.d.

Placental permeability of heparin calculated from equation 1 ($n = 11$) was $8{\cdot}65 \times 10^{-5} \pm 0{\cdot}80 \times 10^{-5}$ mL min^{-1} (g placental tissue)$^{-1}$ (Table 3). The mean total foetal concen-

Table 4. Creatinine permeability measurements on eleven placentae.

Exp. no.	Foetal concn (int. units mL^{-1})	MAUC (int. units min mL^{-1})	FAUC (int. units min mL^{-1})	Wt of placenta (g)	PS (mL min^{-1} g^{-1})
11	4·85	7·29	2·78	83·00	0·013
12	4·68	7·32	4·05	78·60	0·018
14	5·26	7·31	4·94	96·20	0·023
16	4·41	5·43	3·15	91·80	0·021
17	5·47	7·04	3·95	74·50	0·024
19	4·65	6·08	4·26	76·40	0·033
20	4·85	5·72	4·55	81·03	0·051
21	5·43	6·16	3·92	87·50	0·028
22	4·54	4·42	3·71	78·20	0·082
23	4·13	5·03	4·14	80·60	0·057
24	4·67	5·11	3·74	86·40	0·039
Mean	4·68	6·22	3·84	83·11	0·033
s.d.	0·57	1·06	0·62	6·40	0·02
s.e.m.	0·17	0·32	0·18	2·03	0·006

PS = placental permeability.

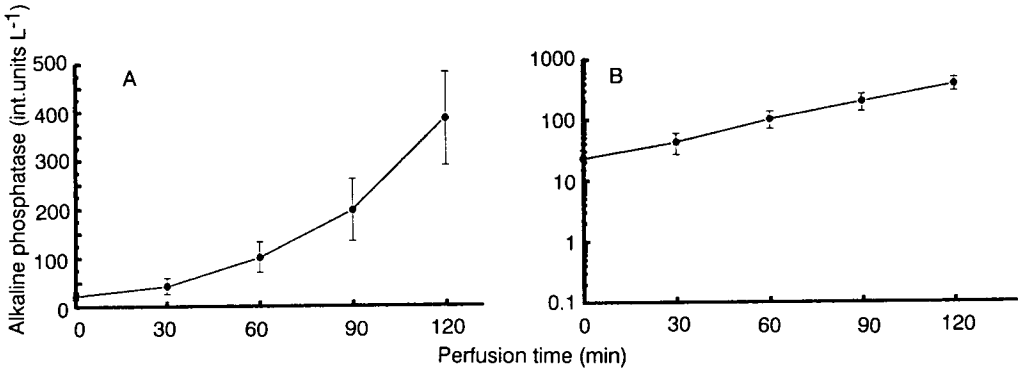

FIG. 2. A. The concentration of alkaline phosphatase in the maternal circulation during 2 h of placental perfusion. B. Values plotted on a logarithmic scale. All values were expressed as mean ± s.e.m. (n = 5).

tration of heparin was 496·43 ± 41·70 int. units. The mean maternal and foetal AUC values in these experiments were 70156·92 ± 1331·95 and 339·92 ± 29·60 int. units min mL^{-1} with mean integrated concentration difference across the placenta of 69817 ± 1340·27 int. units min mL^{-1}. In these experiments placental permeability of creatinine was found to be 0·033 ± 0·006 mL min^{-1} g^{-1} of placenta (Table 4). Clearance of antipyrine was obtained from four experiments under steady-state conditions and was found to be 2·40 ± 0·24 mL min^{-1} with a heparin clearance index of 0·016 ± 0·018. These data suggest that heparin was transferred across the placenta in negligible quantities in contrast to creatinine.

Fig. 2A shows the concentrations of alkaline phosphatase in the maternal perfusate in 5 experiments at 0, 30, 60, 90 and 120 min following perfusion of the placenta. Each experiment shows a slow and steady rise in the concentration of alkaline phosphatase in the maternal perfusate from 0 to 120 min. When alkaline phosphatase concentration was plotted against time on a semi-logarithmic scale, the increment in the concentration of the enzyme was shown to be exponential (Fig. 2B). The foetal concentrations of alkaline phosphatase in these experiments were undetectable. These results indicate that alkaline phosphatase continues to be synthesized by the perfused placental tissue and is subsequently released into the maternal circulation, indicating that the placental tissue remained viable during 120 min of the perfusion.

Fig. 3 shows the concentrations of β-HCG in maternal and foetal circulations at 0 and 120 min, which were obtained from seven experiments. It was evident from Fig. 3A that in the foetal perfusate, concentration of β-HCG increased from undetectable levels to 22·45 ± 6·95 int. units L^{-1} at 120 min. This increase was not statistically significant and was only 0·18% of the maternal concentration at 120 min. In contrast to the foetal perfusate, at time 0 the concentration of β-HCG in the maternal circulation was 2238·14 ± 552·36 int. units L^{-1}. This probably reflects the concentration of β-HCG present in the autologous maternal blood which was used for the preparation of the maternal perfusate. Fig. 3B shows that the maternal concentration of β-HCG increased from 2238·14 ± 552·36 int. units L^{-1} at 0 min to 12376·57 ± 1526·22 int. units L^{-1} at 120 min ($P < 0·001$). Although β-HCG did not rise in a linear fashion in the maternal circulation, it attained high concentrations in the maternal circuit, indicating that this hormone is produced by the trophoblast and is released into the maternal circulation, again suggestive of viability of trophoblastic cells during the experimental period.

Discussion

The foeticidal effects of heparin are poorly understood since heparin is not believed to cross the placenta (Flessa et al 1965). However, such an assumption is not based on any direct evidence, as no data are currently available on the transfer kinetics of heparin in either in-vivo or in-vitro systems.

Our system meets standardized physiological criteria and avoids complications of foetal or maternal metabolism associated with an in-vivo system. Another advantage is that it excludes hepatic biotransformation and renal excretion of drugs, and allows multiple maternal and foetal samples to be obtained. Also the experimental conditions of perfusion can be more accurately controlled than that of an in-vivo system.

Viability of the perfused placental tissue was maintained during the 2 h period as assessed by measurement of β-HCG and heat stable alkaline phosphatase in both maternal and foetal circulations. Geiger et al (1971) have shown negligible amounts of β-HCG (1/500 of maternal concentration) to be present in the foetal circulation, indicating that β-HCG does not cross the placental barrier. However, the same group of investigators found relatively higher concentrations of β-HCG in the foetal circulation in conditions of uteroplacental insufficiency. Although the cause of this phenomenon is unclear, hypoxic damage to the placental membrane was held responsible. Our study indicates that high concentrations of β-HCG were present in the maternal circulation, while negligible amounts were detected in the foetal perfusate after 2 h of perfusion. Similarly, secretion of alkaline phosphatase in high concentration by the trophoblastic cells were found in the maternal circuit, while only traces of the enzyme were present in the foetal perfusate. Although no data are available from an in-vitro perfusion system, Galski et al (1981), using tissue slices from both first trimester and term placenta, showed both synthesis and secretion of the enzyme to take place in the culture media. Moreover, clinical studies have also shown high concentrations of alkaline

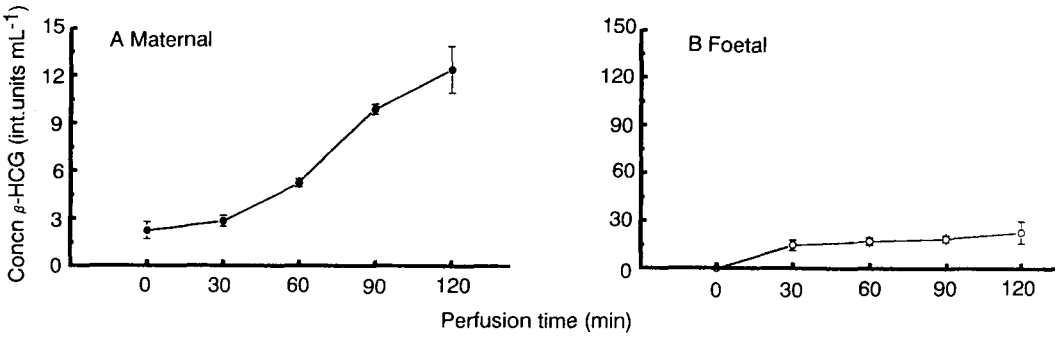

FIG. 3. Maternal (A) and foetal (B) concentrations of β-HCG during 2 h of placental perfusion. In A, data at 30 and 60 min were obtained from 3 experiments, while values at 15, 90 and 120 min were derived from 7 experiments. In B, values at 30 and 90 min were derived from 4 experiments while values at 15, 90 and 120 min were obtained from 7 experiments. All values were expressed as mean ± s.d.

phosphatase to be present in the maternal blood, while enzyme was undetectable in the foetal blood (Adeniyi & Olatunbosun 1984; Contractor et al 1985; Rodin et al 1989), which was also in accordance with the results of this study.

Our study shows that heparin was transferred from the maternal to the foetal circulation, but only in negligible quantities and at a slow rate. Thus this finding is likely to be of no clinical significance if a single intravenous bolus dose of heparin is administered to the mother. Our finding is contradictory to the established view that heparin does not cross the placenta (Flessa et al 1965; Forestier et al 1984; Andrew et al 1985). However, none of these investigators measured heparin concentration either in maternal or cord blood. Their proposition was based on animal or human studies where the transplacental transfer of heparin was excluded from indirect evidence of anti-factor Xa levels and determination of thrombin clotting time of the cord blood. Since the anticoagulant effect of heparin is dose-dependent (Whitfield & Levy 1980), unaltered clotting time cannot exclude transport of small quantities of heparin. Hence, quantitation of heparin concentration in the blood by chemical assay is essential to evaluate its presence. The results of this study show that the transplacental clearance of heparin was 1·16% of antipyrine suggesting that it has a permeability-dependent transfer. In agreement with this observation, studies by Nandakumaran et al (1981), Contractor & Stannard (1983), Ching et al (1987) and Bain et al (1988) have shown the transfer rate of other permeability-dependent substances to be significantly lower than that of antipyrine, such as inulin (7% of antipyrine), benzodiazepine (85%), chlorazepate (20%) and cimetidine (23%).

The poor transfer of heparin is due to at least two factors: its high molecular weight, and its high negative charge. For instance inulin (mol. wt 5000 Da), human serum albumin (mol wt. 69000 Da) and bovine IgG (mol. wt 169000 Da) only cross the placenta in traces (Dancis et al 1976; Contractor & Stannard 1983; Contractor et al 1984; Bain et al 1988). On the other hand substances of lower mol. wt such as creatinine (113 Da), phenytoin (275 Da), diazepam (284·8 Da) and salicylic acid (138 Da) can cross the placenta freely (Levy et al 1975; McAllister 1983; Eaton et al 1985; Shah & Miller 1985).

Another factor which impedes placental transfer of heparin is its hydrophilic property. In agreement with this concept, Illsley et al (1985) and Schneider et al (1985) showed many hydrophilic substances to cross the placenta at slower rates compared with those which are lipid soluble. These authors suggested that placental permeability of hydrophilic molecules was inversely proportional to their molecular weights and was dependent on their coefficient of free diffusion in water (Illsley et al 1985). The results of our study also support this view. For instance, we found that a rise in

Table 5. Comparison of placental permeabilities of hydrophillic substances with that of heparin and creatinine.

Molecules	Mol. wt	D_{37} (cm s^{-1})	Molecular radius (nm)	PS (min mL^{-1} g^{-1})	PS/D (cm g^{-1})
Urea*	60	16·00	0·21	0·047	51
Creatinine	113	11·90	0·29	0·033	46
Erythritol*	122	11·50	0·296	0·032	47
L-Glucose*	180	9·85	0·35	0·023	40
Sucrose*	342	7·50	0·51	0·019	42
Cyanocobalamin*	1355	4·8	0·71	0·014	48
Inulin*	5000	2·6	1·27	0·006	34
Heparin	15000	1·69	2·02	0·00008	85
Albumin*	60000	0·91	3·75	0·0015	24

D_{37} = coefficient of free diffusion in water at 37°C. PS = Permeabilities (g placenta)$^{-1}$.
* PS data obtained from Schneider et al (1985) and Willis et al (1986).

molecular weight of test molecules from 113 (creatinine) to 15000 (heparin) was associated with a marked decline in placental permeability by a factor of 1000. No data are available in the literature on the placental permeability of creatinine or heparin. However, a wealth of information is available from in-vitro and in-vivo studies on the placental permeability of a wide range of hydrophilic substances of variable molecular weights ranging from 60 to 5000 Da (Schneider et al 1985; Willis et al 1986; Bain et al 1990). This is listed in Table 5. Using the above data as standard, placental permeability of creatinine and heparin can be calculated from their molecular weights and are expected to be 0·03 mL min^{-1} g^{-1} and 0·003 mL min^{-1} g^{-1}, respectively. It was interesting to note that in our system, creatinine permeability supported the literature data, but in contrast, in our system the measured permeability of heparin was found to be significantly lower than the expected value. Furthermore, P/D ratio of heparin (the ratio of permeability to coefficient of free diffusion in water) showed a marked decline in relation to other substances (Table 5), indicating that factors other than molecular weight of heparin may account for this difference observed between attained and expected value of placental permeability of heparin. These factors may include experimental error in the measurement of permeability of heparin or its physicochemical characteristics. The possible sources of experimental error could be the failure to quantitate the influence of non-diffusional forces (osmotic or hydrostatic) or transfer of heparin, and the crude estimate of placental exchange area (obtained by using the weight of the perfused tissue). As this difference in the measured and the expected permeability of heparin is greater than 100-fold, i.e. far greater than any conceivable experimental error, this possibility seems unlikely. Furthermore, studies in guinea-pig have indicated that artificial perfusion increases placental permeability (Hedley & Bradbury 1980), but this possibility seems unlikely since the permeability (PS) of creatinine in our system fell within the expected physiological range.

The strong negative charge on the heparin molecule may also be an important factor which accounts for reduced placental permeability of heparin. This view is supported by animal studies. Boyd et al (1976) studying epitheliochorial placenta of sheep, showed that placental permeability of charged molecules was approximately one-hundredth those of uncharged molecules. Similar observations were made in in-vivo studies on human placental permeability (Bain et al 1990). These investigators showed that the transfer of a weakly charged molecule such as chromium ethylenediaminetetraaceticacid was 50% lower than that of lactulose, which had similar molecular weight. They further proposed that the human placenta handled charged molecules as if they were of larger molecular sizes than would be predicted from their simple molecular weights.

Passive diffusion of most hydrophilic compounds across placenta is restricted by the pore diameter of the water-filled extracellular channels (Stulc 1989). The radius of the pore size may cause hinderance to the permeation of molecules of large diameter. In human placenta this pore size has been calculated to be 6 nm (Bain et al 1990). Thus it seems unlikely that the restricted diffusion of heparin across the placental barrier will be due to its molecular size (2 nm as calculated by the Stokes equation) only.

In essence, our results indicate that the placental permeability of heparin was significantly lower than that of creatinine, and factors other than its molecular weight, such as its negative charge, may be important contributors to its reduced permeability across the placenta. In contrast to the conventional view, the finding of our study that heparin can be transferred across the human placenta emphasizes the need for further clinical or in-vitro studies to highlight the mechanisms which account for the deleterious effect of heparin on the foetus.

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