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PLACENTAL TRANSFER OF DIPHENYLHYDANTOIN IN THE GOAT*

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Abstract—Constant blood levels of diphenylhydantoin (DPH) were maintained in pregnant goats by an intravenous infusion. Venous blood was sampled simultaneously from the conscious, unanesthetized mother and fetus during the infusion. Maternal serum levels of DPH were maintained at 14·8 mean, 0·82 S.E. μg/ml. At equilibration, the corresponding fetal level of DPH was 7·6 (0·44 S.E.) μg/ml. When the maternal and fetal sera were equilibrated against each other for 18 hr *in vitro*, the corresponding levels were found to be 13·2 mean, 1·9 S.E. and 6·9 mean, 1·6 S.E. μg/ml respectively. Differences in plasma protein concentration and binding affinity account for the observed maternal-fetal distribution of DPH. Maternal plasma binds more DPH than fetal plasma even when diluted to the same protein concentration as fetal plasma. Antipyrine, which is not appreciably bound to plasma protein at the concentrations studied, distributes equally between maternal and fetal plasma *in vivo*.

ALTHOUGH placental drug transfer has been the subject of a number of reports and review articles,[1–12] confusion about placental permeability of some drugs still exists. The conflicting reports result from inadequate attention to the level of drug achieved in the mother, use of different experimental procedures, and the use of different animal species at different stages of gestation.[7] Much of the data for the placental transfer of drugs in humans has been obtained by analysis of drug levels in the umbilical vessels or in samples of mixed cord blood,[13,14] even though the use of umbilical vessel blood samples as a measure of fetal drug levels may lead to erroneous results.[15,16]

Diphenylhydantoin (DPH) is a drug not infrequently administered to pregnant women. Oh and Mirkin[17] have reported tissue levels of DPH in litters of rats at various times after administration of a single dose of drug. While this technique is widely used,[13,18] the results reflect differential tissue binding as well as placental transfer and so must be interpreted with care.

The present experiments were designed to study the placental transfer of DPH under steady state conditions in the intact, non-anesthetized, pregnant animal. Data will be presented to demonstrate that DPH passively diffuses across the placenta in the goat. However, the steady state level achieved in the fetus is lower than in the mother, due to a difference in the quantity and affinity of the fetal plasma proteins to bind the drug.

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MATERIALS AND METHODS

Angora goats at 110–115 days of gestation were fasted 12 hr before surgery. Atropine (0·7 mg/kg) was injected intravenously 10–20 min before induction of anesthesia with 4% fluothane in a mixture of equal parts nitrous oxide and oxygen. An endotracheal tube was inserted, and anesthesia maintained with a closed system using the same combination of gases except that the fluothane was reduced to 0·5–1·5%. Indwelling catheters were placed in the cranial vena cava of the fetus and the amniotic sac by methods previously described.[3]

The animals were allowed to recover, and the following day a priming dose of 7·3 mg diphenylhydantoin sodium/kg was injected into the maternal external jugular vein, followed by a continuous infusion of 2·92 mg/kg/hr for 2 hr. Blood samples were obtained from mother and fetus simultaneously just prior to administration of DPH and then at varying intervals during the infusion and subsequent 2 hr.

Antipyrine was given simultaneously with DPH in one experiment. Antipyrine was infused at a rate of 47·5 mg/kg/hr following a loading dose of 50 mg/kg.

Binding studies. Plasma protein-binding of DPH *in vitro* was determined in preliminary experiments by equilibrium dialysis according to the method of Anton,[19] and in subsequent experiments utilizing a lucite dialysis chamber* separated by a Visking tubing membrane. In other studies, the apparatus was altered so that the concentration of free drug in equilibrium with the maternal and fetal plasma could be determined simultaneously. A third chamber of 10-ml capacity was placed between the two existing chambers and separated from them by Visking tubing membranes. Each chamber contained a glass bead to aid in mixing. Four ml of fetal and maternal plasma was placed in the two outer chambers, respectively, and 10 ml of 0·05 M phosphate buffer, pH 7·4, in the inner chamber. DPH was added to the central chamber and the system allowed to equilibrate with slow shaking for 18 hr. When the three chambers were filled with phosphate buffer without plasma, DPH was equally distributed throughout them within 12 hr.

Sera obtained from the mother and fetus during the infusion experiments were allowed to equilibrate against each other in a dialysis chamber. All experiments were performed at room temperature.

Analytical. Serum and plasma electrophoreses were performed on cellulose acetate, stained for protein, and quantitated densitometrically.

DPH was assayed by gas–liquid chromatography.[20] Antipyrine was extracted from plasma with ethylene dichloride and an aliquot analyzed by gas–liquid chromatography. A 4-ft long, 0·25-in. diameter stainless steel column was packed with 5% DC 200 on Gas Chrom Q, 80–100 mesh. The column was run at 217° and the peak height response to a flame ionization detector used to quantitate the drug level.

RESULTS

Antipyrine rapidly crossed the placenta and distributed in approximately equal concentrations in maternal and fetal serum (maternal 293 $\pm$ 13 S.E. μg/ml; fetal 264 $\pm$ 7 S.E. μg/ml). The time course of the maternal and fetal DPH serum levels is recorded in Fig. 1. When serum from infused mothers was allowed to equilibrate

* Chemical Rubber Company, Cleveland, Ohio.

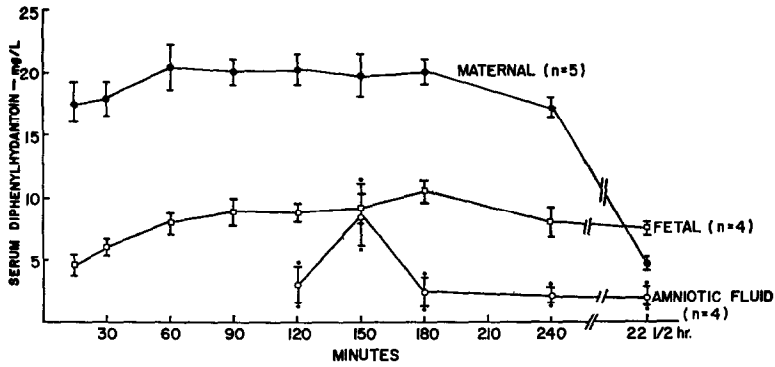

FIG. 1. Time course of maternal and fetal serum and amniotic fluid DPH levels during constant infusion of DPH (2·92 mg/kg/hr) following a loading dose of 7·3 mg/kg. The infusion is discontinued at 120 min.

in vitro against that of the fetus, no significant change from the level of DPH *in vivo* was seen (Table 1). Similarly, when DPH was allowed to equilibrate with fetal and maternal serum *in vitro*, the concentration of DPH in the maternal serum was approximately twice that in the fetus (16·4 vs. 9·8 μg/ml). The unbound fraction of DPH ranged from 3 to 5 per cent in the maternal serum and from 6 to 9 per cent in fetal serum when the total DPH level was in the therapeutic range (Table 2).

TABLE 1. SERUM LEVELS OF DPH—EQUILIBRIUM STUDIES *in vivo* AND *in vitro**

	Diphenylhydantoin (μg/ml)		
Serum	30–240 min *in vivo*		After 18 hr equilibration *in vitro*
Maternal	Mean	14·8	13·2
	S.E.	0·82†	1·9
Fetal	Mean	7·6	6·9
	S.E.	0·44	1·6
No. of samples		40	5

* Samples of maternal and fetal serum were removed between 30–240 min after initiation of the DPH infusion and dialyzed against each other for 18 hr (see text for full explanation).

† S.E., standard error of the mean.

The total protein content of fetal serum was 3·11 ± 0·10 g/dl while that of the mother was 6·36 ± 0·16 g/dl. This difference was due primarily to a reduction in the albumin and γ-globulin fractions (Table 3).

Freundlich isotherms for the binding of DPH to fetal and maternal plasma and to maternal plasma diluted to the same protein content as found in fetal plasma are shown in Fig. 2. At a given free drug concentration, maternal plasma binds more

TABLE 2. DIPHENYLHYDANTION CONCENTRATION OF FETAL AND MATERNAL PLASMA AFTER EQUILIBRIUM DIALYSIS AGAINST 0·05 M PO_4, pH 7·4*

Plasma	Diphenylhydantoin		
	Total (μg/ml)	Unbound (μg/ml)	Unbound (%)
Material	16·8	0·74	4
	15·8	0·48	3
	16·5	0·81	5
	40·0	5·9	15
	48·2	8·3	18
Fetal	8·6	0·74	9
	8·3	0·48	6
	12·4	0·81	7
	21·2	5·9	28
	30·2	8·3	28

* A three-chamber dialysis apparatus was used for these studies. The inner chamber contained DPH in buffer and the outer chambers maternal and fetal plasma. Values are mean of duplicate determinations.

TABLE 3. PROTEIN CONTENT OF GOAT BLOOD

		Total protein (g/dl)	Electrophoretic fractions (g/dl)				
			Albumin	α_1	α_2	β	γ
Material serum		6·3	2·96	0·50	0·63	1·00	1·20
		5·9	2·84	0·65	0·76	0·41	1·41
		6·8	2·18	0·61	0·68	0·54	2·31
		6·7	2·65	0·60	0·67	0·53	2·27
		5·8	2·88	0·63	0·75	0·52	1·39
		6·7	2·84	0·47	0·88	0·47	1·61
	Mean	6·36	2·72	0·58	0·72	0·57	1·69
	S.E.	0·16	0·11	0·03	0·00	0·08	0·18
Plasma		6·0	2·34	0·60	0·60	0·78	1·92
Fetal serum		3·2	2·05	0·44	0·35	0·35	0·00
		3·3	1·95	0·49	0·10	0·50	0·20
		3·1	1·91	0·52	0·15	0·37	0·24
		3·1	1·77	0·52	0·15	0·37	0·24
		3·4	1·98	0·48	0·54	0·13	0·07
		2·6	1·90	0·49	0·10	0·36	0·18
	Mean	3·11	1·92	0·49	0·21	0·34	0·15
	S.E.	0·10	0·04	0·04	0·05	0·00	0·04
Plasma		3·2	1·61	0·32	0·06	0·41	0·41

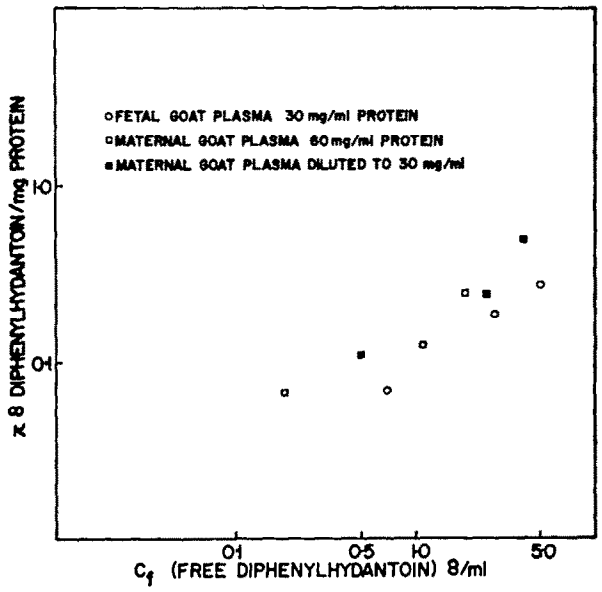

FIG. 2. Freundlich isotherms for binding of DPH to fetal, maternal and diluted maternal plasma. The log of micrograms of DPH bound per milligram of protein is plotted against log free DPH concentration.

DPH, even when diluted to contain the same total protein concentration as fetal plasma.

Plasma DPH levels at 5, 10 and 15 min after the start of the infusion were utilized in the calculation of an apparent placental transfer coefficient (P) for DPH in the goat. The coefficient was calculated using three different functions of drug concentration: (1) an apparent P was calculated using total serum DPH levels; (2) the calculation was corrected for the equilibrium maternal-fetal distribution of DPH determined *in vivo* at later time periods during the infusion; (3) free drug concentrations in fetal and maternal plasma at the DPH concentration encountered in the infusion experiments were estimated from the drug-binding experiments *in vitro* and utilized in the calculation of P. The results of these calculations are shown in Table 4.

TABLE 4. APPARENT PLACENTAL TRANSFER COEFFICIENT BASED ON:*

Total drug concn.	Total concn. corrected for equilibrium	Free drug concn.
$Pt = -\ln [1 (C_2/C_1)]$	$Pt = -\ln [1 - (C_2/C_1 \cdot R)]$	$Pt = -\ln (fc_1 - f'c_2/fc_1)$
	$R = 0{\cdot}522$	$f = 0{\cdot}05$ $f' = 0{\cdot}08 - 0{\cdot}11$
$P = 0{\cdot}0154$	$P = 0{\cdot}0273$	$P = 0{\cdot}0418$

* P = placental transfer coefficient; t = time; C_1 = serum concentration of DPH in mother; C_2 = serum concentration of DPH in fetus; f = unbound fraction of DPH in mother; f′ = unbound fraction of DPH in fetus, R = equilibrium ratio. See text for further explanation.

DISCUSSION

Theoretical calculations of placental transfer coefficients, P, have been based on Fick's law under the simplifying conditions that the concentration of diffusing solute on the maternal side of the placental barrier remains constant over the course of the experiment.[12] As many drugs are not equally distributed across the placenta at equilibrium, a correction factor (R) was introduced into the calculation of P for the substances.[1,12] The implicit assumption in the use of R is that it is independent of drug concentration. Justification for this assumption depends upon the mechanism by which the unequal concentrations of drugs across the placenta are maintained at equilibrium. Differential protein-binding of drug to fetal and maternal plasma protein has been proposed as one such mechanism.[21] As drug bound to plasma proteins is not diffusable, only free drug in plasma is available to drive diffusion. It is obvious that using total plasma drug concentrations leads to a marked underestimation for P and DPH, a significant fraction of which is bound to plasma protein. The free drug concentration may or may not be a constant fraction of the varying total drug concentration in plasma and thus, R, in fact, may or may not be constant during the course of the experiment. The results shown in Table 4 suggest that, over the range of concentrations encountered in the present experiments, R is not constant. The values of P obtained using R are not the same as those utilizing the free drug concentration in fetal and maternal plasma.

In the calculation of P it is important to use the concentration of diffusable drug, i.e. that fraction of the total concentration which is free in solution. For DPH, this fraction varies with total drug concentration. Therefore, although the use of R (equilibrium ratio of fetal to maternal drug concentration) leads to an improvement over the use of total drug concentration, it underestimates P, due to an increased fraction of drug being bound to fetal plasma at lower concentrations.

The observation that equilibrium dialysis *in vitro* gives the same distribution of drug between fetal and maternal serum as observed *in vivo* between fetus and mother indicates that unbound DPH freely crosses the placenta by simple diffusion. The observed distribution of DPH between fetal and maternal serum can be explained by the differential binding of DPH in the two fluids. This is in contrast to antipyrine which is not significantly protein bound[22] and distributes equally across the placenta. Maternal serum binds a greater fraction of the drug than fetal serum even when diluted to a protein concentration equal to fetal serum, demonstrating a qualitative as well as quantitative difference in binding of DPH.

If unbound DPH is freely diffusable across the placenta, the concentration of unbound DPH in fetal and maternal plasma is equal at equilibrium. Furthermore, if one assumes that only the unbound drug is pharmacologically active, then the fetal and maternal receptor sites are exposed to the same concentration of DPH, even though the concentration of total DPH in fetal plasma is one-half that in the mother. This phenomenon must be taken into consideration when concentrations of total drug are used to study placental transport or used as an index of the potential for toxic effects to the fetus. For example, Mirkin[14] has reported that peak fetal rat DPH brain levels were twice those of maternal brain. From these data one cannot determine whether the increased concentration is due to non-specific binding or an increase in unbound DPH in equilibrium with receptor sites.

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