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The effect of pregnancy in humans on the pharmacokinetics of stable isotope labelled phenytoin

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1 To investigate the mechanism of the fall in steady-state plasma phenytoin concentration relative to drug dose that occurs during pregnancy, single dose pharmacokinetic studies with stable isotope labelled phenytoin were carried out at different stages of pregnancy, and 2 to 4 months post-natally, in five epileptic women receiving regular oral therapy with the drug.

2 Steady-state apparent plasma clearances of phenytoin (dose/steady-state concentration) correlated closely with simultaneous plasma clearances of the intravenous stable-isotope drug (measured as dose/AUC) suggesting that the patients were compliant with therapy when their phenytoin dosage requirement increased during pregnancy, and that the oral drug was fully bioavailable.

3 In retrospect, two of the five subjects were probably studied too early post-natally for phenytoin elimination kinetics to have returned to non-pregnant values. Despite this, (i) the mean ± s.d. $t_{½}$ for phenytoin was statistically significantly shorter in pregnancy than post-natally (31 ± 14 *vs* 39 ± 28 h), (ii) the mean ± s.d. whole plasma clearance was also statistically significantly greater (0.025 ± 0.012 *vs* 0.021 ± 0.013 l kg^{-1} h^{-1}) and (iii) the mean ± s.d. V_{max} for phenytoin elimination was statistically significantly greater in pregnancy (1170 ± 600 mg day^{-1}) than post-natally (780 ± 470 mg day^{-1}). Although the mean ± s.d. apparent K_m was higher in pregnancy (18.2 ± 8.4 mg l^{-1}, expressed in terms of whole plasma drug concentrations, compared with 10.2 ± 7.4 mg l^{-1} post-natally), the difference was not statistically significant. However, if the apparent K_m value was expressed in terms of plasma water phenytoin concentrations the difference (pregnant 2.50 ± 0.85 mg l^{-1}: post-natally 1.16 ± 0.65 mg l^{-1}) was statistically significant.

4 Human pregnancy appears to result in an increased capacity to eliminate phenytoin.

Keywords pharmacokinetics pregnancy phenytoin stable-isotope Michaelis-Menten parameters

Introduction

Several studies have shown that epilepsy tends to worsen during pregnancy (e.g. Knight & Rhind, 1975), although not all investigations have supported this conclusion (Schmidt, 1982). It is now known that, in women, plasma concentrations of the major antiepileptic drugs, including phenytoin, often fall relative to drug dose as pregnancy progresses, and then return to prepregnancy values over the weeks or months following childbirth (Eadie *et al.*, 1977; Lander *et al.*, 1977; Nau *et al.*, 1982). This changed relationship between plasma drug concentration and drug dose probably contributes to any tendency to increased epileptic seizures that occurs in pregnant women. Adjustment of anticonvulsant dosage to maintain plasma drug concentrations at prepregnancy values throughout pregnancy seems to improve seizure control during this period.

Several explanations have been offered for

the fall in plasma anticonvulsant concentrations relative to drug dose which occurs during pregnancy. The possibility that epileptic women may comply with the prescribed drug doses less adequately when pregnant has been raised (Nau *et al.*, 1982), possibly because of fears that the therapy may harm their babies. It is clearly difficult to obtain reliable data on this question. Others have suggested that drug absorption may be impaired in pregnancy (Ramsay *et al.*, 1978), but our earlier kinetic study (Lander *et al.*, 1984) showed that oral phenytoin was reasonably well absorbed in pregnant women, at least for the first dose of the drug taken. It is possible that the increasing fluid volume of the pregnant woman's body, and that of the foetus, may in a sense dilute the drug dose, resulting in falling maternal plasma drug concentrations; this factor seems unlikely to account for the magnitude of the effect observed (Eadie *et al.*, 1977). Another possibility, as Mygind *et al.* (1976) suggested, is that anticonvulsant clearance increases in pregnancy, probably because of increasing metabolism in the maternal and foetal livers (and perhaps in the placenta). Since anticonvulsant plasma concentration changes persist for weeks or months after delivery, the occurrence of placental and foetal contributions to drug metabolism is not a sufficient explanation for the fall in drug levels in pregnancy, though it might contribute. We have recently shown in *in vitro* studies that the human placenta of subjects not pre-exposed to phenytoin has little or no capacity to metabolize this drug (Kluck *et al.*, 1988).

To clarify further the mechanism(s) underlying the fall in plasma anticonvulsant concentrations relative to drug dose during pregnancy, sequential pharmacokinetic studies in the same women when pregnant and when not pregnant would be desirable. Ethical considerations preclude the giving of unnecessary drugs to pregnant women. Therefore such sequential pharmacokinetic studies would have to be carried out in treated epileptic patients, but in these women it would be unjustifiable to omit therapy for several days while carrying out a kinetic study following the most recent drug dose. Tracer quantities of radioactive anticonvulsants cannot be given for kinetic studies in pregnancy because of hazards for the fetus. However the use of stable isotope labelled tracer doses of anticonvulsants for such kinetic studies, while routine anticonvulsant therapy is continued, would be ethically justifiable, and is technically feasible. We here report such studies carried out during pregnancy, and post partum, using [^{13}C], [^{15}N]-labelled phenytoin, in an attempt to determine the mechanisms of the known fall in plasma drug concentrations that occurs relative to phenytoin dose in pregnant women.

Methods

Subjects studied

The studies were carried out in five informed consenting pregnant women, following approval of the investigational protocol by the Ethics Committees of the University of Queensland and the Royal Women's Hospital, Brisbane. Personal details of the subjects and of their pregnancies and details of their epilepsies are provided in Table 1.

Study design

The original intention was that each subject should be studied once in each trimester of pregnancy, and once at least 2 months post-partum. In practice, patients presented to the Royal Women's Hospital too late in pregnancy for any studies in the first trimester to be carried out. It proved impractical to carry out studies at exactly the same stages of pregnancy and of the postnatal period in all subjects. The times the studies were carried out, in relation to the duration of pregnancy, and the subjects' body weights, regular daily doses of phenytoin and mean plasma phenytoin concentrations at the times of each study, are shown in Table 2.

On the morning of each study the patient took by mouth as a single dose her normal 24 h intake of sodium phenytoin less 50 mg. At the same time she received intravenously a nominal 50 mg of stable isotopic phenytoin (5,5'-diphenylhydantoin-[^{13}C]:90%;[^{15}N]:99%, purchased from CEA, Centre d'Etudes Nucleaires de Saclay, France). This was prepared in the Manufacturing Dispensary of Royal Brisbane Hospital to British Pharmacopoeal specifications for phenytoin i.v. The volume of the intravenous injection was measured accurately, and the phenytoin content of the residue of the solution in the syringe was assayed, so that the actual dose of stable isotopic drug given was known (Table 2). Thereafter, patients followed their ordinary daily routines while venous blood samples were collected for drug assay as near as practicable to 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 24, 32, 48, 56 and 72 h from the time of drug administration. Patients resumed their routine daily phenytoin dosage after 24 h. All urine passed by the patients over the 72 h after administration of the labelled drug was collected for measurement of phenytoin and its metabolites.

Table 1 Personal details of the subjects studies

Subject	*Age (years)*	*Pregnancy outcome*	*Epilepsy*	*Therapy*
J	29	spontaneous vaginal delivery: baby—high arched palate	temporal lobe epilepsy: incompletely controlled in pregnancy	phenytoin
E	22	spontaneous vaginal delivery: normal baby	temporal lobe epilepsy for 7 years: full seizure control during pregnancy	phenytoin; iron; folate
B	26	spontaneous vaginal delivery: normal baby	partial epilepsy for 7 years: fully controlled throughout pregnancy	phenytoin; diazepam; iron; folate
P	24	Caesarian section: normal baby	generalised epilepsy for 7 years: well controlled during pregnancy	phenytoin; iron; folate
M	21	Caesarian section: normal baby	generalised epilepsy for 9 years: decreased seizure control in pregnancy but therapy changed from valproate at outset of pregnancy	phenytoin

Table 2 Patient details at times of kinetic studies

Subject	*Stage of pregnancy (weeks)*	*Weight (kg)*	*Regular phenytoin dose (mg day^{-1})#*	*Mean whole plasma phenytoin (mg l^{-1})*	*Mean % phenytoin unbound in plasma*	*Isotopic phenytoin dose (mg)*
J	36	55.8	396	7.55	15.2	37.5
	+9	50	322	7.09	11.1	50
E	31	69.4	396	6.45	11.7	44
	37	72.6	460	8.18	12.2	50
	+8	62	368	5.15	9.2	50
B	23	62.5	359	21.85	17.7	46.9
	30	67.5	359	12.58	12.2	50
	+16	62	368	15.64	17.6	49.5
P	24	75.1	423	8.26	16.2	48.75
	34	79.2	477	7.83	17.6	47.5
	+16	76	396	13.19	12.0	49.4
M	20	70	331	16.27	12.7	48.75
	31	78.5	368	12.10	14.4	48.75
	+15	68	368	35.19	11.8	47.5

#Values are for phenytoin itself, not sodium phenytoin.

Phenytoin assay

Plasma (1.0 ml) was acidified with 0.2 M hydrochloric acid (0.1 ml) and extracted with chloroform (5.0 ml) containing the internal standard (5-(4-methylphenyl)-5-phenylhydantoin; TPH) 1.0 mg l^{-1}. After centrifugation the aqueous layer and precipitated proteins were aspirated to waste, the chloroform solution was transferred to a clean tube, and the solvent removed under a gentle air stream. The residue was dissolved in *N*,*N*-dimethylacetamide (40 μl) and alkylated by the addition of trimethylphenylammonium hydroxide (1.0 M in methanol, 10 μl) and 1-iodopropane (10 μl). The mixture was vortex agitated, centrifuged, and 1–3 μl of the clear supernatant applied to the GC-MS.

The instrument was a Finnigan model 3300 GC-MS, with a pyrex column (1.5 m × 2 mm i.d.) packed with 3% OV-101, and held at 215° C. The carrier gas was methane (18 ml min^{-1}) which also served as the reagent gas for the chemical ionization mass spectrometer. The Finnigan model 6110 data system controlling the mass spectrometer permitted continuous monitoring of the ions at m/z values 337.3, 340.3, and 351.3 corresponding to the protonated molecular ions of phenytoin, [2-^{13}C-1,3-di^{15}N]-phenytoin and TPH respectively.

Plasma standards spiked with various concentrations of phenytoin and labelled phenytoin were carried through the entire procedure to establish calibration curves for both analytes. The calibration curves showed excellent linearity over the concentration ranges 0.1–10.0 μg ml^{-1} (labelled drug) and 0.5–50 μg ml^{-1} (unlabelled drug). The coefficient of variation was approximately 10% at all concentrations and the limit of quantification was 0.1 μg ml^{-1} for both analytes.

Plasma protein binding studies

After the completion of the drug concentration measurements sufficient plasma remained to allow determination of the plasma protein binding of phenytoin at the time of each pharmacokinetic study.

Plasma samples (1 ml) were spiked with [4-^{14}C]-phenytoin (47.05 nCi $nmol^{-1}$, New England Nuclear) and dialysed overnight at 37° C against a pH 7.4 buffer (150 mM sodium chloride, 10 mM sodium phosphate). Duplicate aliquots (0.4 ml) of plasma and dialysate were counted with 5 ml scintillant (PCS II, Amersham).

The mean value of the duplicate assays for the unbound phenytoin percentage in plasma at the time of each pharmacokinetic study is shown in Table 2. These values were used to convert plasma drug concentrations to the corresponding plasma water drug concentrations.

Pharmacokinetic analysis

Plasma stable isotope phenytoin concentration-time data were studied by (i) multiple compartmental linear pharmacokinetic analysis and (ii) multiple compartmental analysis with elimination of the drug from the central compartment described as Michaelis-Menten kinetics. Both approaches required the use of three compartment models in nearly all instances to obtain adequate curve fits to the data. The equation fitted for the linear kinetic analysis was of the form

$$C = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} + C_3 e^{-\lambda_3 t}$$

where C = concentration of isotopic drug at time t, and λ_1, λ_2 and λ_3 are hybrid rate constants.

Curve fitting (linear model) was carried out on an Apple IIe microcomputer using a least squares iterative method based on the Fehlberg modification of the Marquardt algorithm (available from Stemsoft Pty Ltd, Brisbane, Australia).

The Michaelis-Menten elimination kinetic approach (three compartment model—Figure 1) involved simultaneous fitting of the stable isotope concentration-time data to the following differential equations:

$$dC(1)/dt = k_{21}.\,C(2) + k_{31}.\,C(3) - k_{12}.\,C(1) - k_{13}.C(1) - \frac{V_{max}.C(1)}{K_m + C(1) + C(n1)}$$

$$dC(2)/dt = k_{12}.\,C(1) - k_{21}.\,C(2)$$

$$dC(3)/dt = k_{13}.\,C(1) - k_{31}.\,C(3)$$

with initial conditions

$$C(1)_0 = \text{dose}/V_1$$

$$C(2)_0 = 0$$

$$C(3)_0 = 0$$

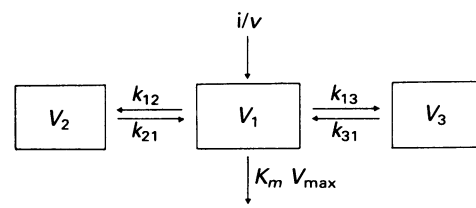

Figure 1 The three compartment model used in the pharmacokinetic studies.

where $C(1)$, $C(2)$ and $C(3)$ refer to concentrations of isotopic drug in compartments 1, 2 and 3 respectively, $C(n1)$ refers to concentration of non-isotopic drug in compartment 1 (the central compartment), V_{max} is the maximum velocity of elimination, K_m is the apparent Michaelis constant (since the actual drug concentration at elimination sites is not measured), k_{12} etc. refer to intercompartmental transfer rate constants and V_1 is the volume of the central compartment. This curve fitting was also carried out on an Apple IIe microcomputer, but employing a modification of the program 'Multi' (Yamaoka *et al.*, 1981). This program also employs an iterative least squares approach using the simplex algorithm, with numerical integration being carried out by the Runge-Kutta-Gill method. For the purpose of the iterations, knowledge of the patients' actual simultaneous daily phenytoin doses under steady-state conditions (Table 2) permitted the setting of realistic lower limits to the calculated V_{max}, and correspondingly to the apparent K_m.

For the linear compartmental analysis, half-lives were derived from the slowest calculated elimination rate constant for each study as ln2/rate constant. Area under the plasma concentration-time curve (AUC(0,t)) was determined to the last measured data point by trapezoidal rule integration, and AUC(t,∞) was calculated as C_t/λ_3. AUC was the arithmetic sum of AUC(0,t) and AUC(t,∞). Whole plasma clearance was calculated for the isotopic drug as dose/AUC, and for the non-isotopic drug apparent clearance as daily dose/steady state plasma drug concentration. The volume of distribution of isotopic drug was calculated by dividing the clearance by the terminal elimination rate constant.

For the nonlinear (i.e. Michaelis-Menten) compartmental analysis, parameters were as calculated directly in the iterations. Apparent K_m values were expressed as mg l^{-1} of whole plasma or of plasma water ($\times$ 1000/252, to convert to μmol l^{-1}). V_{max} values were expressed in mg day^{-1}, the figures derived from the iterations being multiplied by the corresponding volume of the central compartment and by 24 to obtain the V_{max} values shown.

Statistical evaluation of the results employed two factor analysis of variance, carried out on an IBM microcomputer running the CSS statistics package.

Results

With the unexplained exception of Subject B, plasma protein binding of phenytoin was lower in late pregnancy than post-natally. The mean $\pm$ s.d. percentage unbound for the five women was 14.4 $\pm$ 2.4 in pregnancy, and 12.3 $\pm$ 3.1 afterwards.

The clinicians responsible for the patients' care adjusted phenytoin doses during and after pregnancy with the intention of maintaining plasma drug concentrations within predetermined ranges known to maintain control of the patients' seizures. The maximum phenytoin dose increases prescribed during pregnancy (both total amount of drug and amount relative to body weight) and steady-state phenytoin apparent clearance (dose/C_{ss} ratio) changes, are shown in Table 3. The phenytoin dose and steady-state drug apparent clearance had not always returned to baseline values by the time the post-partum kinetic study was done. In retrospect, it appeared that the studies done before 4 months post partum might not reflect the basal state. In all subjects the phenytoin dose had to be increased to maintain plasma phenytoin concentrations, and the drug's steady-state apparent clearance rose during pregnancy.

The time courses of the whole plasma concentrations of labelled and non-labelled phenytoin over the periods of a study in one subject are shown in Figure 2. This indicates that relatively little fluctuation occurred in the total plasma phenytoin concentrations over the individual study period, so that control of the patient's epilepsy should not have been compromised by the studies. A set of plasma concentration-time curves for stable-isotope labelled phenytoin given to one subject (M) at two different stages of pregnancy, and in the post-natal period, is

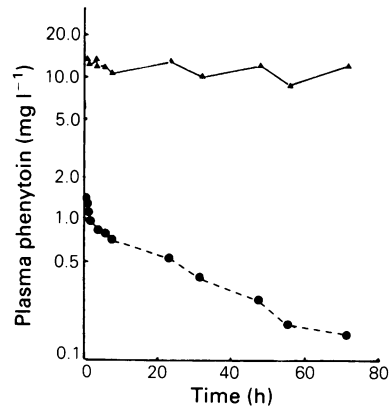

Figure 2 Semi-logarithmic plot of the time-courses of plasma concentrations of non-isotopic (▲) and isotopic (●) phenytoin during a pharmacokinetic study in Subject P, at 16 weeks post-partum. Only alternate data points are shown in the first 10 h, to avoid congestion.

Table 3 Maximum changes in phenytoin dose and steady-state phenytoin apparent clearance (dose/C_{ss} ratio) during pregnancy, and the final recorded post-partum phenytoin dose and apparent clearance. Doses are expressed in terms of phenytoin itself, and not phenytoin sodium

	Dose (*mg day*$^{-1}$)		*Dose increase*		C_{ss} *clearance* (*l kg*$^{-1}$ *h*$^{-1}$)		*Final post-partum*		
Subject	*minimum*	*maximum*	(*mg day*$^{-1}$)	(*mg kg*$^{-1}$ *day*$^{-1}$)	*minimum*	*maximum*	*dose* (*mg day*$^{-1}$)	*clearance#* (*l kg*$^{-1}$ *h*$^{-1}$)	*stage* (*weeks*)
J	322	460	138	2.16	0.016	0.061	322	0.026	+9
E	322	488	166	2.01	0.027	0.047	368	0.048	+8
B	359	423	64	0.24	0.013	0.021	368	0.015	+16
P	368	515	147	0.44	0.028	0.037	396	0.016	+16
M	276	451	175	1.19	0.006	0.022	368	0.006	+15

#The discrepancies between these apparent clearance values and those shown in Table 4 are due to single routine laboratory plasma concentration values being used in the above calculations, and averaged values over the duration of the isotopic studies measured in a research laboratory in Table 4. The value for J may reflect a laboratory error in a single measurement.

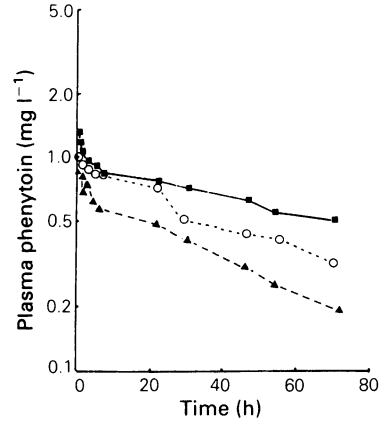

Figure 3 Semi-logarithmic plots of the time-courses of plasma concentrations of isotopic phenytoin following similar intravenous doses of the labelled drug given to subject M at 20 (○) and 31 (▲) weeks of pregnancy, and at 15 (■) weeks post natally. Only alternate data points are shown in the first 10 h, to avoid congestion.

shown in Figure 3. The behaviour of the concentrations of stable isotope labelled drug suggests that kinetic analysis of such data should be feasible. In practice the linear and Michaelis-Menten curve fitting to the sets of stable isotope labelled phenytoin concentration-time data appeared adequate. Coefficients of determination for the curve fitting were above 0.958 for all data sets and were 0.990 or greater for approximately half the data sets in the whole study.

The relevant linear pharmacokinetic parameters for the individual subjects calculated from curve fitting to a three compartment model (or a two compartment model for subject B and for one set of data from subject P) are set out in Table 4. Simultaneous steady-state apparent clearances (dose/C_{ss} ratios) for unlabelled phenytoin are also shown, for comparison. Two factor analysis of variance demonstrated statistically significant differences between the terminal half-lives of phenytoin while the subjects were pregnant (mean ± s.d. 30.79 ± 13.87 h), and after childbirth (39.16 ± 27.75 h) ($F = 8.887$, d.f. = 1, 4; $P = 0.0401$). Similarly, mean ± s.d. isotope labelled phenytoin plasma clearance in pregnancy (0.0246 ± 0.0117 l kg^{-1} h^{-1}) was higher than mean post-partum clearance (0.0206 ± 0.0126 l kg^{-1} h^{-1}), the differences being statistically significant ($F = 15.968$; d.f. 1, 4; $P = 0.0172$). If the clearance was expressed in terms of plasma water volumes, the difference was no longer significant (mean ± s.d. pregnant 0.1774 ± 0.0891 l kg^{-1} h^{-1}: post-natally 0.1876

Table 4 Pharmacokinetic parameters obtained from fitting a three compartment linear model to the stable isotope labelled phenytoin concentration-time data. Steady state apparent whole plasma clearance of non-isotopic phenytoin over the study period is shown for comparison. CL′ refers to plasma water phenytoin clearance

Subject	*Stage of pregnancy (weeks)*	λ_3 (h^{-1})	$t_{1/2}$ (h)	*AUC* (mg l^{-1} h)	V (l kg^{-1})	*CL* (l kg^{-1} h^{-1})	*CL′* (l kg^{-1} h^{-1})	CL_{ss} (l kg^{-1} h^{-1})	r^2
J	36	0.048	14.53	15.743	0.895	0.043	0.281	0.039	0.994
	+9	0.037	18.73	38.726	0.697	0.026	0.232	0.038	0.990
E	31	0.041	17.03	17.106	0.912	0.037	0.317	0.037	0.978
	37	0.037	18.84	21.310	0.878	0.032	0.265	0.032	0.994
	+8	0.025	27.51	20.574	1.556	0.039	0.426	0.048	0.996
B	23	0.013	52.12	60.061	0.940	0.013	0.071	0.011	0.962
	30	0.018	37.88	48.896	0.825	0.015	0.124	0.018	0.986
	+16	0.020	33.98	52.374	0.745	0.015	0.086	0.016	0.985
P	24	0.027	25.77	24.283	0.993	0.027	0.165	0.028	0.998
	34	0.029	23.74	20.315	1.010	0.030	0.168	0.033	0.970
	+16	0.025	27.73	38.978	0.668	0.017	0.139	0.016	0.997
M	20	0.015	47.80	65.199	0.738	0.011	0.095	0.012	0.994
	31	0.018	39.38	42.428	0.830	0.015	0.112	0.016	0.994
	+15	0.008	87.84	116.333	0.759	0.006	0.054	0.006	0.996

± 0.1494 l kg^{-1} h^{-1}: F = 0.3299; d.f. 1, 4; P = 0.596).

The whole plasma clearance of stable isotope phenytoin found in each study is plotted against the simultaneous steady-state apparent clearance (dose/C_{ss} ratio) of non-isotopic phenytoin in Figure 4. The two parameters correlated well (r = 0.945), the paired values in most instances being close to equal.

The relevant pharmacokinetic parameters calculated by fitting a three compartment model with Michaelis-Menten type elimination from the central compartment to the concentration-time data are shown in Table 5. Two factor analysis of variance indicated that the mean ± s.d. apparent K_m value in pregnancy, expressed in terms of whole plasma drug concentration (18.20 ± 8.43 mg l^{-1}), was not statistically significantly greater (F = 4.992; d.f. 1, 4; P = 0.0888) than the mean value post-partum (10.19 ± 7.42 mg l^{-1}). However if the parameters were expressed in terms of plasma water phenytoin concentration, the difference became statistically significant (pregnant: 2.5 ± 0.85 mg l^{-1}: postnatally 1.16 ± 0.65 mg l^{-1}: F = 25.142; d.f. 1, 4; P = 0.0087). The mean ± s.d. V_{max} value in pregnancy (1174.6 ± 596.4 mg day^{-1}) was statistically significantly greater (F = 11.675; d.f. = 1, 4; P = 0.0276) than the mean V_{max} after pregnancy (777.7 ± 470.7 mg day^{-1}).

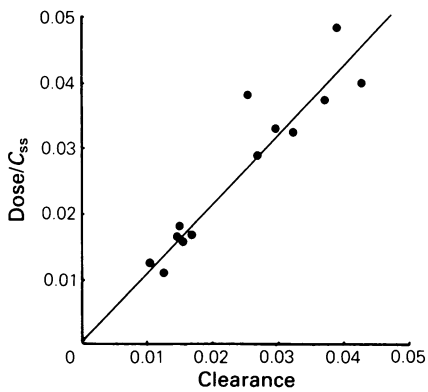

Figure 4 Regression for simultaneous values of phenytoin apparent clearance measured as dose/steady-state concentration of non-isotopic drug and as dose/AUC for the pulsed dose of isotopic drug. The equation for the regression line is y = 0.0016 + 0.8509x (r = 0.945: P < 0.001).

Discussion

The technique used for the present study, employing tracer doses of stable isotope labelled phenytoin, has advantages over other methods of investigating antiepileptic drug kinetics in human pregnancy. It permits accurate delineation of phenytoin kinetics without compromising continuity of therapy and seizure control and does not expose mothers or offspring to radioactivity. The simultaneous concentrations of the stable isotopic drug and the unlabelled drug can be measured separately, each with great sensitivity, by mass spectrometry, as has been shown previously (van Langenhoave *et al.*, 1980). An important requirement of such labelled dose

Table 5 Pharmacokinetic parameters obtained by fitting a three compartment model with Michaelis-Menten elimination from the central compartment to the isotope labelled phenytoin concentration-time data. K_m' refers to the apparent K_m expressed in terms of plasma water phenytoin concentration

Subject	*Stage of pregnancy (weeks)*	V_1 *(l)*	K_m *(mg l^{-1})*	K_m' *(mg l^{-1})*	V_{max} *(mg day^{-1})*	r^2
J	36	21.78	4.35	0.66	580.2	0.974
	+9	19.44	13.09	1.45	758.5	0.987
E	31	44.69	24.65	2.88	2014.5	0.973
	37	26.39	21.13	2.58	2023.5	0.995
	+8	42.34	21.46	1.97	1583.6	0.992
B	23	43.72	11.50	2.04	572.7	0.958
	30	36.00	25.00	3.05	945.9	0.987
	+16	26.82	7.27	1.28	523.4	0.983
P	24	42.76	19.94	3.23	1372.8	0.992
	34	42.19	19.97	3.52	1607.1	0.973
	+16	23.91	7.12	0.85	634.1	0.994
M	20	26.71	16.40	2.08	615.6	0.991
	31	24.02	17.01	2.45	839.1	0.991
	+15	25.43	2.01	0.24	389.0	0.989

methods, if they are to produce valid metabolic and pharmacokinetic data, is that there be no isotope effect on metabolism. The [^{13}C],[^{15}N]-labelled phenytoin used in the present study has been demonstrated to show pharmacokinetic equivalence with unlabelled phenytoin (Browne *et al.*, 1981).

To obtain satisfactory curve fits (all coefficients of determination in excess of 0.958), the plasma stable isotope phenytoin concentration-time data were interpreted in terms of a three compartment model, whether elimination was considered to follow linear or Michaelis-Menten processes. The need to invoke so many pharmacokinetic compartments may have resulted from measuring many early data points during the drug's distributional phases.

It is known that the plasma protein binding of phenytoin is slightly decreased in later human pregnancy (free fraction 12.4 ± 1.1% as compared with the normal value of 9.9 ± 0.8%—Perucca *et al.*, 1981). The limited plasma protein binding studies that were possible in the present investigation also showed reduced plasma protein binding of phenytoin during the pregnancies. Over the whole plasma concentration range 4–30 mg l^{-1} the plasma protein binding of phenytoin is not concentration-dependent (Hooper *et al.*, 1973). Therefore it was considered justifiable to use the measured free fraction of phenytoin in plasma at a particular stage of pregnancy to convert all whole plasma phenytoin levels at that stage of pregnancy to the corresponding plasma water drug concentrations.

The main interest in the study lay in the kinetic parameters reflecting drug elimination, *viz.* the terminal $t_{½}$, clearance, V_{max} of elimination and apparent Michaelis constant. It has been accepted that the elimination of phenytoin is better described by Michaelis-Menten than by linear kinetics (Gerber & Wagner, 1972). For such a drug the calculated half life and clearance will vary with the dose, and with the plasma concentration range over which these two parameters are measured. In the present investigation the kinetic studies in any one subject could not always be carried out at identical steady-state plasma phenytoin concentrations. Consequently it is difficult to be certain of the significance of the half-life and whole plasma clearance differences between pregnancy and the post-partum period found in individual subjects. For the whole group, differences in these two linear kinetic parameters proved statistically significant at the 5% level of confidence, though clearances expressed in terms of plasma water volumes did not differ. The calculation of the Michaelis-Menten parameters, measured on the basis of studies carried out in the presence of steady-state concentrations of non-isotopic phenytoin, involved the assumption that isotopic and non-isotopic drug behave as competing substrates for an enzyme system or systems displaying identical V_{max} and K_m properties towards both. This assumption necessitates the introduction of a term for steady-state non-isotopic drug concentration into the denominator of the Michaelis-Menten component of the

equation describing kinetic events in compartment 1 (as was also done by Browne *et al.*, 1985). Iterative fitting of V_{max} and K_m values to the ordinary Michaelis-Menten equation can involve the practical problem of the values of both parameters being increased almost in parallel so that the elements fitted in successive iterations ultimately become virtually a V_{max}/K_m ratio (Metzler & Tong, 1981). To reduce the risk of this happening the calculation of the Michaelis-Menten parameters in the present study also involved the setting of an upper limit for the apparent K_m (30 mg l^{-1}) and a lower limit for V_{max}. The latter corresponded to the actual daily phenytoin dose the patient received at the time of each study (carried out under steady-state conditions). Further, in the present study an additional term (the non-isotopic drug concentration) was present in the denominator of the Michaelis-Menten equation. This term varied from one data point to the next, and was roughly an order of magnitude greater than the simultaneous isotopic drug concentration. This additional term may have lessened the risk of V_{max} and K_m increasing in parallel during the iterations.

Clinical and practical considerations made it difficult for the kinetic studies to be undertaken at identical stages of pregnancy and the postnatal period in all subjects. Nevertheless, despite the unexplained different behaviour of the parameters in Subject J, the mean V_{max} for phenytoin elimination proved higher in pregnancy than post-natally, and in all four subjects in whom the Michaelis-Menten parameters were estimated twice in pregnancy, the V_{max} was higher later in pregnancy than earlier in pregnancy. There tended to be a greater fall in V_{max} from maximum measured pregnancy values, when the parameter was remeasured 4 months post-partum than when it was remeasured 2 months post-partum. It is therefore possible that the timing of the post-partum kinetic studies may not have allowed the maximum increases in V_{max} that occurred in pregnancy to be demonstrated, since the post-natal V_{max} values may not have always had time to fall to pre-pregnancy basal ones by the time they were measured. The post-partum apparent K_m values similarly may not have always been the subject's basal ones and the difference from the K_m values measured during pregnancy may therefore not have revealed the maximum changes which occurred in this parameter. The mean post-partum apparent K_m value, expressed in terms of whole plasma drug concentration, (10.19 ml l^{-1}) may appear high in relation to some published figures for this parameter e.g. 4 mg l^{-1} (Richens, 1974). However, the K_m as measured may not be a basal non-pregnant value, and in any event it is not discordant with some published estimates of the K_m value in non-pregnant persons e.g. 11.5 ± 5.0 mg l^{-1} (Martin *et al.*, 1977), 9.43 ± 2.62 mg l^{-1} (Jung *et al.*, 1980).

Although the present study design has proved to have certain shortcomings, the investigation has thrown light on several aspects of the pharmacokinetics of phenytoin in pregnant women. There was good agreement between steady-state non-isotopic phenytoin apparent clearance (i.e. dose/C_{ss} ratio) during pregnancy, and simultaneous whole plasma clearance of pulsed doses of stable isotope phenytoin. Such close agreement would be very unlikely if the increase in steady-state phenytoin apparent clearance during pregnancy was artefactual, and due either to non-compliance with prescribed dosage or to failure to absorb the full phenytoin dosage ingested. In either of these events, calculated steady-state non-isotopic drug apparent clearance should have exceeded isotopic drug clearance. Taken in conjunction with the earlier finding that the first oral dose of phenytoin taken during pregnancy was virtually fully bioavailable in a different group of pregnant epileptics (Lander *et al.*, 1984), the present result suggests that the increased phenytoin dose/concentration ratio during pregnancy is a real phenomenon, and due to some alteration in the disposition of the drug. Phenytoin is eliminated mainly by metabolism (Glazko *et al.*, 1969). The increase in the maximum velocity of phenytoin elimination during pregnancy found in the present study suggests that the capacity of the maternal-foetal unit to biotransform the drug is increased during pregnancy. This increase in ability to metabolise phenytoin appears a sufficient explanation for the tendency of the drug's plasma concentrations to fall, relative to drug dose, as pregnancy progresses. As mentioned earlier (and also as found in the present study), phenytoin apparent clearance does not return promptly to pre-pregnancy values when the foetal and placental components of the maternal-foetal unit are abruptly subtracted from the unit at childbirth (Eadie *et al.*, 1977). This suggests that the pregnant woman's increased metabolic capacity towards phenytoin is largely resident in the maternal tissues. Possibly the hormonal changes of pregnancy induce the maternal liver mono-oxygenase system and increase its capacity to biotransform phenytoin. It is possible that the folate taken during pregnancy by three of the five subjects may have contributed to the increased phenytoin elimination. However, the increased elimination did not occur in these three women only. Folate

doses as low as 1 mg a day may be associated with a fall in plasma phenytoin concentrations (Berg *et al.*, 1983), but the present subjects were not prescribed more than 0.3 mg day^{-1}. Other data (Eadie *et al.*, 1977) indicate that folate administration is unlikely to be the full explanation of the altered phenytoin disposition that occurs in human pregnancy.

The findings of the present study make it difficult to be sure whether the K_m for overall phenytoin elimination is really altered during pregnancy. The drug has more than one known pathway of oxidative metabolism and the individual pathways may have different K_m values. If the overall K_m is altered it may merely mean that all the alternative metabolic pathways are not changed in capacity to the same extent during pregnancy. It is known that the urinary excretion of the main phenytoin metabolite *p*-hydroxy phenytoin does not increase in human pregnancy despite the altered plasma drug concentration:dose relationship (Landon & Kirkley, 1979; Kochenour *et al.*, 1980), raising the possibility that conversion of phenytoin to another metabolite becomes increasingly important in this circumstance.

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