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Stereoselective Metabolism and Disposition of the Enantiomers of Mephenytoin during Chronic Oral Administration of the Racemic Drug in Man[1]

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ABSTRACT

Küpfer, A., P. V. Desmond, S. Schenker and R. A. Branch: *Stereoselective metabolism and disposition of the enantiomers of mephenytoin during chronic oral administration of the racemic drug in man. J. Pharmacol. Exp. Ther.* **221:** 590–597, 1982.

The stereospecific disposition of the enantiomers of mephenytoin has been investigated during chronic oral administration of racemic mephenytoin (23 μmol/kg/day) for 14 days in four normal subjects. On days 1 and 11, 5 μCi of [^{14}C]-S-mephenytoin and 45 μCi of [^{3}H]-R-mephenytoin were administered concomitantly with unlabeled racemic mephenytoin. Plasma mephenytoin concentrations reached a peak on the 2nd day of administration, then declined to a plateau between the 7th and 14th day. Mephenytoin was metabolized to two major metabolites. Firstly, 4-hydroxylation of the phenyl ring [3-methyl-5-(4-hydroxy phenyl)-5-ethylhydantoin; 4-OH-M) yielded a metabolite which was rapidly eliminated in urine to quantitatively account for almost half of the racemic dose administrered. This amount remained constant during chronic therapy. The second metabolc route was demethylation to 5-phenyl-5-ethylhydantoin (PEH). The renal clearance for this metabolite was lower than its synthesis rate; thus, the initial low plasma level of PEH accumulated until it achieved a 10:1 ratio in excess of parent drug after 12 days of therapy. As urinary clearance of PEH remained constant, daily urinary excretion increased in a similar time profile to that observed in plasma. The initial radiotracer study confirmed stereoselective 4-hydroxylation of S- but not R-mephenytoin. S-mephenytoin was rapidly and quantitatively converted to 4-OH-M and excreted in urine within the dosage interval. The tritium label on R-mephenytoin was slowly excreted in urine in the form R-PEH and minor unidentified phenolic metabolites other than 4-OH-M or 4-OH-PEH. The second radiotracer study confirmed that the stereospecificity of hydroxylation of S-mephenytoin persisted and suggested that the rate of synthesis of R-PEH increased. The possibility of autoinduction was supported by a study in a single subject, who received only R-mephenytoin on day 1 and 11, but racemic mephenytoin on days 2 to 10 and 12 to 14. In this subject, apparent oral clearance of R-mephenytoin increased from 33 to 106 ml/min. In conclusion, with daily oral administration of racemic mephenytoin, S-mephenytoin is eliminated as 4-OH-M within each dosage interval and will not accumulate to make a significant contribution to the therapeutically effective hydantoin, whereas R-mephenytoin is converted to R-PEH which is the major contributant to the therapeutic response.

Mephenytoin (Mesantoin; Sandoz Pharmaceuticals, Hanover, NJ) is a 5,5-disubstituted hydantoin (3-methyl-5-phenyl-5-ethylhydantoin) used in antiepileptic treatment in man (Troupin *et al.*, 1976, 1979). Carbon-5 of the hydantoin ring represents a center of asymmetry leading to two stereoisomeric (enantiomeric) forms of the molecule (fig. 1). The 1:1 mixture of the two enantiomers (racemate) is presently used in clinical practice. In man, major routes of metabolism are aromatic hydroxylation of the position 4 of the phenyl ring to produce 4-OH-M, which is subsequently conjugated and rapidly eliminated in urine (Troupin *et al.*, 1976; Lynn *et al.*, 1979; Küpfer *et al.*, 1980) and, secondly, demethylation to PEH which is the major hydantoin found in plasma during chronic mephenytoin therapy. As neither of these metabolic transformations influence the center of asymmetry, metabolites have the same enantiomeric configuration as their parent enantiomer.

In a previous study, the stereospecificity of these routes of metabolism have been investigated after single dose administration of racemic mephenytoin (Küpfer *et al.*, 1981). The approach used in that study was to separate the enantiomers, label each with either ^{14}C or ^{3}H and then reconstitute a pseudoracemic mixture. After single oral dose administration, there was a marked difference in urinary metabolic profile between the two enentiomers. S-mephenytoin according to the Cahn *et*

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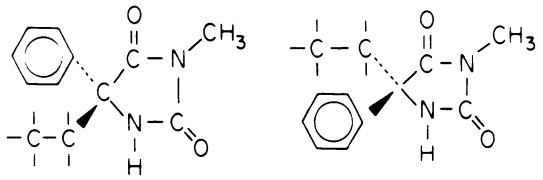
ABBREVIATIONS: 4-OH-M, 3-methyl-5-(4-hydroxyphenyl)-5-ethylhydantoin; PEH, 5-phenyl-5-ethylhydantoin; GLC, gas-liquid chromatography; LSC, liquid scintillation counting; HPLC, high-performance liquid chromatography; PPH, 5-phenyl-5-prophylhydantoin; DMSO, dimethylsulfoxide.



l-MEPHENYTOIN (R)-ENANTIOMER d-MEPHENYTOIN (S)-ENANTIOMER

Fig. 1. Chemical structure of the enantiomers of mephenytoin.

al. (1956) was rapidly excreted as 4-OH-M, whereas R-mephenytoin contributed to the major proportion of PEH formed. This latter metabolite was only slowly excreted in urine. The enantiomeric differences in urinary excretion suggested that the R but not S-enantiomer would be expected to accumulate during chronic therapy and provide the major contribution to circulating hydantoin levels.

The objective of the present study was to investigate this hypothesis by measuring the stereochemical composition of mephenytoin and its metabolites in plasma and urine during chronic oral therapy in normal subjects. The enantiomeric contributions to urinary metabolites were evaluated by administering enantiomeric dual-radiolabeled, pseudoracemic mephenytoin at the start and again after 11 days of drug administrtion.

Materials and Methods

[^{14}C]-S- and [^{3}H]-R-mephenytoin pseudoracemate. Radiolabeled racemic PEH was synthesized using either the Bucherer-Berg synthesis with [^{14}C]KCN for the ^{14}C-labeling of the position 4 carbon in the hydantoin ring (Henze and Isbell, 1954) or by catalytic hydrogenation with $^{3}H_2$ of 5-(4-bromophenyl)-5-ethylhydantoin for ^{3}H-labeling of the 4 position hydrogen in the phenolic ring (Küpfer *et al.*, 1981). Racemic [^{3}H]- and [^{14}C]PEH were separated into the two enantiomers by fractional crystallization of their diastereomeric brucine salts (Sobotka *et al.*, 1932). Methylation at position 3 of the hydantoin was the final step of the mephenytoin synthesis (Grimmer 1969). Details of procedures used are reported elsewhere (Küpfer *et al.*, 1981). The optical rotations for S- and R-mephenytoin were $[\alpha]_D^{25} = +105°C$ and $-104°C$, respectively. This implies a relative optical purity of 100 and 99.5%, respectively. Unlabeled racemic mephenytoin was isolated from Mesantoin tablets (Sandoz Pharmaceuticals) and purified by crystallization from hot ethanol in water (1:10).

The dual radiolabeled mephenytoin for oral administration contained 5 μCi of [^{14}C]- S-mephenytoin, 45 μCi of [^{3}H]-R-mephenytoin and additional amounts of unlabeled S- and R-enantiomers were added to achieve a final total dose of 23 μmol/kg (1 μmol of mephenytoin is equivalent to 218 μg) of a 1:1 S- to R-enantiomeric ratio. For oral administrtion, pseudoracemic mephenytoin or unlabeled racemic mephenytoin was dissolved in 4 ml of propylene glycol then further diluted in 100 ml of water. For i.v. administration. [^{3}H]-R-mephenytoin (38 μCi) was dissolved in 4 ml of water, propylene glycol and ethanol (5:4:1). The solution was sterilized by millipore-filtration. Tests for sterility and for the absence of pyrogens were performed by local manufacturers of i.v. preparations according to the USP guidelines.

Experimental protocol in man. After providing informal consent for the protocol which had been approved by the University Ethics Committee, four normal, non-smoking, drug-free, male volunteers (age 23–39 years, weight 64–78 kg) participated in the study. Each subject was screened by a history, physical examination and routine laboratory tests. The enantiomerically radiolabeled mephenytoin pseudoracemate (5 μCi of ^{14}C, 45 μCi of ^{3}H and 23 μmol/kg of unlabeled drug) was administered orally in predissolved solution at 8:00 A.M. to fasting subjects on days 1 and 11. On days 2 to 10 and 12 to 14, each subject received 23 μmol/kg of predissolved unlabeled racemic mephenytoin at 8:00 A.M. after a light breakfast. Blood was collected before the dosage interval and complete urine samples were obtained at 24-hr intervals during the whole experimental period. On days 1 and 11, serial plasma and urine samples were collected over the dosage interval. After 14 days, when drug administration was discontinued, several blood samples were obtained from each subject at 4- to 5-day intervals over 3 weeks to measure the terminal half-life of mephenytoin and PEH.

Combined single dose and i.v. study of R-mephenytoin alone. Three months after the oral study, a single subject received unlabeled R-mephenytoin (11.5 μmol/kg) orally together with i.v. [^{3}H]-R-mephenytoin (38 μCi) on days 1 and 11 of the study. The subject received 23 μmol/kg of mephenytoin racemate per day on days 2 to 10 and 12 to 14, receiving the dose on day 2, 30 hr after the initial dose, but thereafter receiving each dose at 24-hr intervals. Plasma and urine samples were collected at the same time intervals as described above.

Analysis of mephenytoin and its metabolites. GLC was used to measure mephenytoin, PEH and 4-OH-M in urine samples using methods previously described (Küpfer *et al.*, 1980, 1981). The ratio of [^{14}C]-S- and [^{3}H]-R-enantiomers of these three compounds in urine were determined from double isotope LSC of the respective HPLC fractions (Küpfer *et al.*, 1981).

Plasma concentrations of mephenytoin and PEH were determined by a modification of the GLC method used to measure urinary samples. The internal standard, PPH (8 nmol) was added to 1 ml of plasma and acidified with 100 μl of 3 N hydrochloric acid. Organic extraction was performed with 6 ml of ether and the supernatant was evaporated under a gentle stream of air. The residue was dissolved in 200 μl of DMSO (dried over calcium hydride) and 50 μl of 1-iodopropane was added to form the propylated derivative. The reaction was initiated by the addition of 40 mg of sodium hydride, suspended in 2 ml of heptane. The *in situ* formation of the DMSO carbonion (Coreybase) permits perpropylation of hydantoins within 30 min under constant vortexing at room temperature. The reaction was stopped by the addition of 4 ml of water and the perpropylated derivatives were extracted into 4 ml of ether. The organic solvent was evaporated in a separate test tube. The dry residue was dissolved in 20 μl of methanol and an aliquot of 5 μl was injected into the gas chromatograph. A Perkin Elmer gas chromatograph model 3920 B was used equipped with a 1.8-m glass column (internal diameter, 2 mm) packed with 3% S.E.-30 on Varaport 30 100/200 mesh (Varian Associates, Palo Alto, CA). Peak detection was obtained with a nitrogen specific thermionic detector (P/N-detector, Perkin Elmer) connected to an electronic integrator Varian model CDS 211-C for peak area measurements. At 180°C isothermal conditions, the following retention times were found for the perpropylated hydantoins: mephenytoin at 2.4 min, PEH at 5.3 min and the internal standard PPH at 6.5 min. Linear calibration graphs with r values ranging from 0.997 to 0.999 were obtained between 50 and 2500 nmol/ml in blank plasma for both mephenytoin and PEH. The y intercepts were not significantly different from zero in any instance of four replicates.

Plasma protein binding of the R- and S-enantiomers of mephenytoin and PEH. Plasma protein binding was measured by equilibrium dialysis using the various radiolabeled enantiomers at separate occasions in the concentration range of 20 to 500 nmol/ml. A Dianorm equilibrium dialyzing system (Spectrum Medical Industries Inc., Los Angeles, CA) with 1.5 ml chamber size Teflon cells was used together with Spectrapore dialysis membranes (MW cutoff, 12,000–14,000; Fisher Scientific Products, Pittsburgh, PA). Plasma samples were dialyzed against phosphate buffer (66 mM at pH 7.4). In a previous experiment, it was established that equilibrium was reached not earlier than after 120 min at 37°C. Serial dialysis experiments were carried out over 240 min at the same water bath temperature.

Pharmacokinetic analysis. After i.v. administration of [^{3}H]-R-mephenytoin to one subject, an apparent volume of distribution of ^{3}H was estimated by a back extrapolation of the stable plateau concentration between 6 and 30 hr, based on the assumption that in man, like the dog, the volume of distribution of mephenytoin and PEH are the same (Küpfer and Bircher, 1979; Küpfer *et al.*, 1977). An apparent volume of distribution unlabeled mephenytoin after oral administration

was calculated from:

$$V_D(\text{apparent}) = \frac{D}{\text{AUC} \times \beta} \quad (1)$$

where D is the dose, AUC the area under the plasma concentration curve extrapolated to infinity, and β is the terminal rate constant of elimination.

Total plasma clearance (Cl_T) of R-mephenytoin was calculated from:

$$Cl_T = \frac{\text{Dose}}{\text{AUC}} \quad (2)$$

where the AUC was extrapolated to infinity after a single dose or the AUC during the dosage interval was used during chronic therapy. Renal clearance (Cl_R) of PEH was calculated from:

$$Cl_R = \frac{U \cdot V}{P} \quad (3)$$

where U is urine concentration of PEH, V is urine volume, and P is the mean plasma concentration of PEH during the urine collection interval.

Results

Plasma mephenytoin and PEH concentrations. In four normal, drug-free subjects, plasma concentration of racemic mephenytoin rose rapidly to a peak at 1 hr after the first oral dose administration of predissolved racemic drug (fig. 2, left panel). Subsequently, it declined rapidly with an apparent half-life of 5.3 ± 0.5 hr (mean ± S.E.M.) over the first 6 hr before reaching an apparent plateau level by the dosage interval. With repeated daily doses, trough plasma concentrations rose to a peak by the second day; then tended to decrease to a stable steady-state level of approximately 5 nmol/ml (fig. 3; table 1). On day 11, blood samples were drawn at similar time intervals to day 1. Even though plasma concentrations of mephenytoin were higher at each time point after drug administration on day 11 in comparison to day 1, the extent of parent drug accumulation during chronic therapy was minimal (fig. 2, right panel). The major difference in plasma mephenytoin concentration time profile was that plasma concentrations 24 hr after mephenytoin administration were significantly lower than 6 hr after administration on day 11 ($P < .05$), but not on day 1. In contrast to plasma levels of parent drug, initial plasma levels of PEH were below 2 nmol/ml after a single dose (fig. 3). However, they rose during subsequent chronic drug administration to reach an apparent plateau by day 11. At this time, there was a ratio of nearly 10:1 between plasma PEH and mephenytoin levels (fig. 3). After discontinuation of mephenytoin administration, plasma PEH levels decreased with a half-life of 141 ± 9 hr (fig. 4).

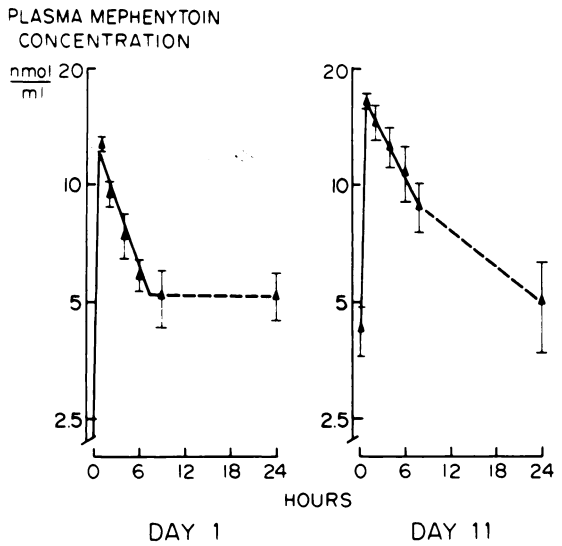


Fig. 2. Plasma concentration of mephenytoin measured by GLC during the dosage interval on days 1 and 11 of chronic racemic mephenytoin adminstration (n = 4, mean ± S.E.M.).

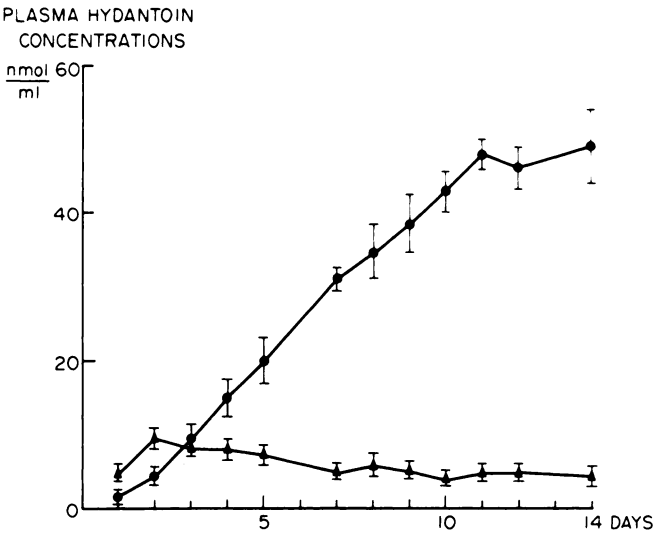


Fig. 3. The change in plasma concentration of mephenytoin (▲) and PEH (●) with respect to time. Plasma levels were measured using GLC before dosing during chronic daily administration of racemic mephenytoin n = 4, mean ± S.E.M.).

TABLE 1

Mephenytoin and PEH pharmacokinetic parameters during last 3 days after 14 days of daily mephenytoin administration (mean ± S.E.M.)

Mephenytoin plasma concentration (nmol/ml)	4.8 ± 0.72
PEH plasma concentration (nmol/ml)	49.1 ± 4.9
Urinary clearance of PEH (ml/min)	4.4 ± 0.3
Terminal plasma half-life of PEH (hr)	141 ± 9

Urinary mephenytoin and its metabolites. Mephenytoin was not excreted in urine to any appreciable extent, with total recovery of parent drug being less than 2% of the dose per day. 4-Hydroxymephenytoin was a major urinary metabolite, accounting for 45 ± 3% (mean ± S.E.M.) of total racemic dose administered after the first dose of mephenytoin. The daily urinary excretion of 4-OH-M remained constant at this high level throughout multiple dose treatment (fig. 5). In contrast, the urinary elimination of PEH had a similar profile to the rise in plasma PEH concentration (fig. 4). The daily excretion rate was below 5 μmol/24 hr for the first 3 days then rose to a plateau of approximately 230 μmol/24 hr from day 11 onward. Urinary clearance of PEH was low and was 4.4 ± 0.3 ml/min between 11 and 14 days (table 1). Other potential metabolites of mephenytoin, such as isomeric phenols or ethyl side chain hydroxylated products, were not detected in urine within the limits of sensitivity of the GLC assay used and therefore did not account for more than 5 μmol/24 hr. Acid hydrolysis of urine will convert hydantoic acid to the hydantoin and leave glucuronide and sulfate conjugates. Assays for PEH in urine before and after acid hydrolysis indicated that 33% of PEH was metabolized by these routes in one subject, whereas only 11.5 to 12.5% (range) was metabolized in the other three subjects.

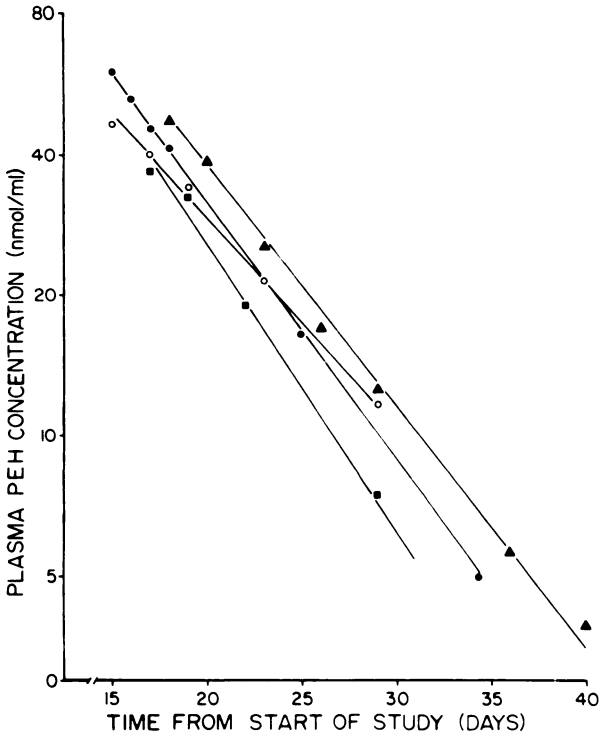


Fig. 4. Plasma concentrations of PEH in each of the four subjects after discontinuation of chronic mephenytoin therapy.

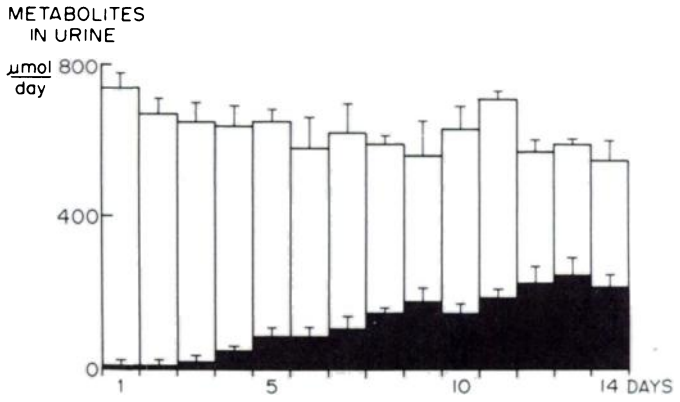


Fig. 5. The daily excretion of 4-OH-mephenytoin (in open bars) and PEH (in solid bars) measured by GLC during chronic administration of racemic mephenytoin (n = 4, mean ± S.E.M.).

Enantiomeric composition of urinary mephenytoin metabolites. [^{14}C]-S-mephenytoin and [^{3}H]-R-mephenytoin were administered with racemic mephenytoin on days 1 and 11 in order to evaluate the stereospecificity of mephenytoin metabolism before and after chronic administration of the racemic drug. After the first dose, the urinary excretion rate of [^{14}C]-S-enantiomer was rapid, with a peak rate 1 hr after oral administration and a subsequent half-life of 3.5 ± 0.4 hr (fig. 6, left panel). This rapid urinary excretion rate resulted in 95 ± 3% of the administered ^{14}C-radioactivity dose being recovered in 24 hr and essentially complete recovery within 48 hr (fig. 6). The initial rapid absorption, excretion and quantitative recovery of ^{14}C were similar on day 11 (figs. 5 and 6) with 91 ± 1.1% of the ^{14}C-label being recovered. HPLC confirmed that the [^{14}C]-S-enantiomer was present in the form of 4-OH-M.

In contrast to the S-enantiomer, urinary tritium recovery from [^{3}H]-R-mephenytoin was only 8.7 ± 0.5% of the first dose administered during the first 24 hr, slightly more the next day, then the rate of urinary excretion declined slowly (fig. 7). Before the second radiolabeled dose on day 10, the urinary excretion

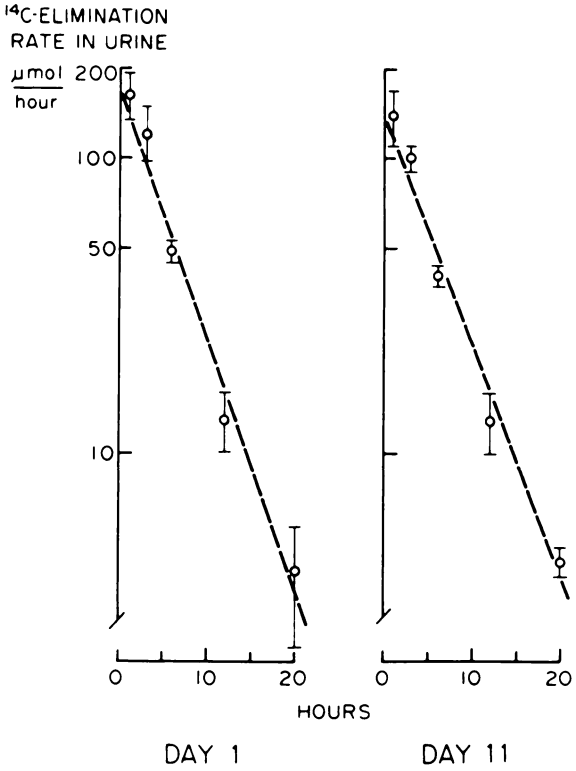


Fig. 6. Urinary excretion rate of ^{14}C after administration of [^{14}C]-S-mephenytoin on days 1 and 11 of chronic racemic mephenytoin administration (n = 4, mean ± S.E.M.).

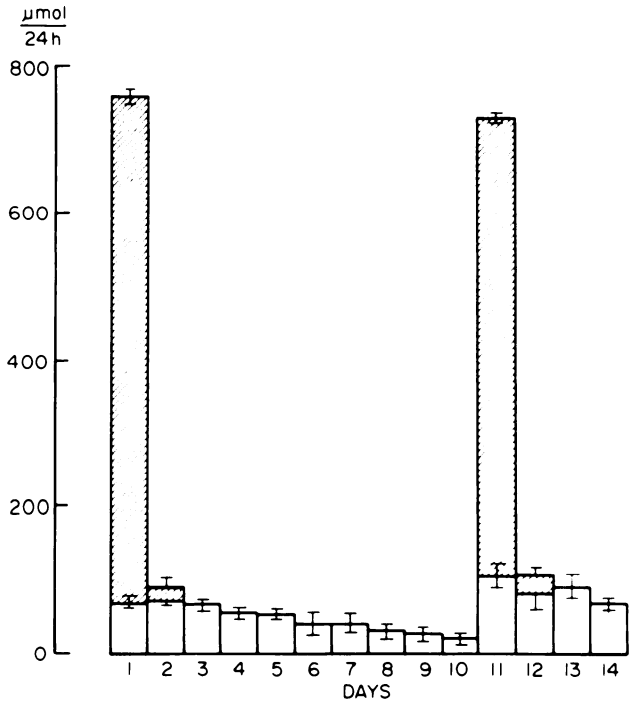


Fig. 7. The daily urinary excretion of ^{14}C (in cross-hatched bars) and ^{3}H (in open bars) after oral administration of [^{14}C]-S-mephenytoin and [^{3}H]-R-mephenytoin on days 1 and 11 and unlabeled racemic mephenytoin on days 1 to 14 (n = 4, mean ± S.E.M.).

of ^3H was 2.6% of the dose given in 24 hr. After the second dose at day 11, the sum of the expected tritium from the first dose, together with the tritium from the second dose, was essentially the same as the initial 24-hr urinary excretion of tritium after the first dose. The subsequent rate of excretion of tritium was followed for a further 4 days, during which unlabeled mephenytoin administration was still continued. During this time period, the profile was similar to that after the first dose.

Analysis with HPLC indicated a different time course profile of PEH urinary elimination in comparison to total tritium elimination (fig. 8). Thus, urinary excretion of R-PEH started at a low level, increased to a peak by the 5th day and declined by the 10th day. After the second dose of radiolabel, the initial appearance of R-PEH was more rapid, reaching a peak by the second day. Although plasma levels of PEH were low, there was an equal recovery of both R- and S-PEH in urine after the first radiolabeled drug administration. However, after PEH accumulation in plasma by day 10, essentially all the PEH appearing in the urine after the second dose of radiolabeled drug was in the form of the R-enantiomer.

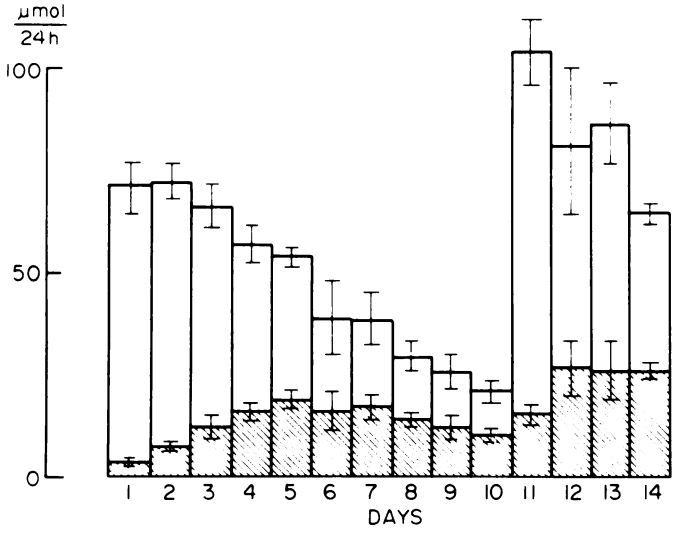


Fig. 8. The daily urinary excretion of total ^3H (in open bars) and [^3H] PEH (in cross-hatched bars) after administration of [^3H]-R-mephenytoin on days 1 and 11 and unlabeled racemic mephenytoin on days 1 to 14 (n = 4, mean ± S.E.M.).

Combined oral and i.v. administration study of R-mephenytoin. It was not possible to determine the enantiomeric composition of mephenytoin and PEH in plasma because the relatively low doses of radioactivity of ^3H and ^{14}C did not permit accurate, quantitative double isotope LSC analysis. To circumvent this and to provide an indication of the rate of conversion of R-mephenytoin to R-PEH, a single subject received half the racemic dose in the form of R-mephenytoin orally on days 1 and 11. The opportunity was also taken to determine pharmacokinetic parameters by administering a tracer dose of [^3H]-R-mephenytoin i.v. on days 1 and 11 simultaneously with oral administration of unlabeled drug (fig. 9). After the first dose, plasma concentrations of R-mephenytoin initially rose rapidly to a peak, then declined biexponentially. Plasma R-PEH levels were low during the first 8 hr, but rose to be almost equal to R-mephenytoin levels by 30 hr. Plasma tritium levels reflected an initial distribution phase for the first 6 hr, then reached a plateau as the sum of both R-mephenytoin and R-PEH. The time that the tritium levels achieved a plateau coincided with the onset of the terminal exponential of plasma mephenytoin. Assuming that man is similar to the dog in having equal volumes of distribution for mephenytoin and PEH (Küpfer and Bircher, 1979; Küpfer *et al.*, 1977), the volume of distribution measured from the tritium plateau back extrapolated to the time of drug administration was 1.46 liters/kg. This was in good agreement with the apparent volume of distribution of unla-

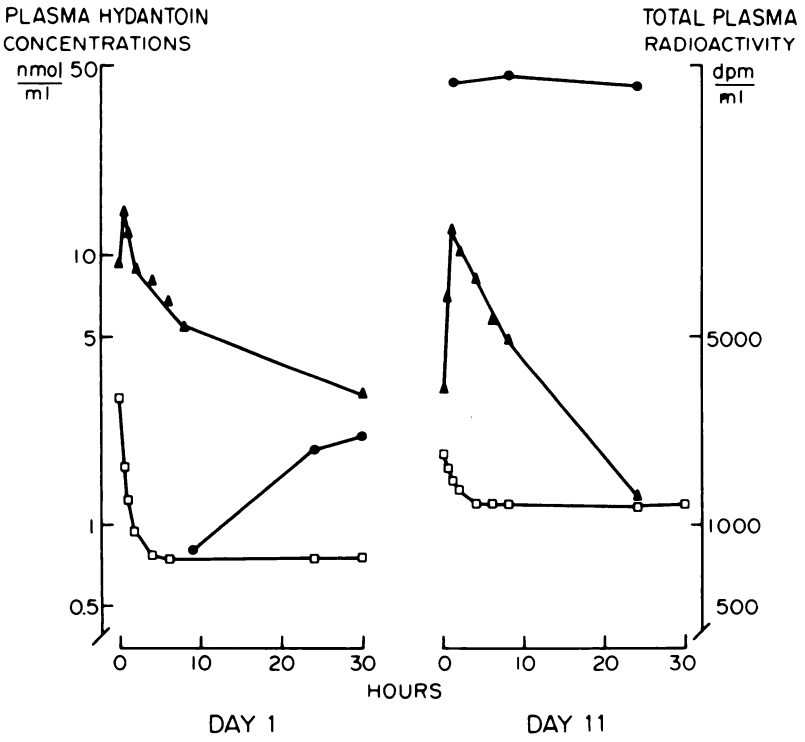


Fig. 9. Plasma concentrations of mephenytoin (▲), PEH (●) and ^3H (□) during a dosage interval on days 1 and 11 in a single subject. This subject received R-mephenytoin orally simultaneously with [^3H]-R-mephenytoin i.v. on days 1 and 11 and received racemic mephenytoin on days 2 to 10 and 12 to 14.

beled R-mephenytoin given orally, suggesting complete gastrointestinal absorption. Thus, plasma clearance could be estimated from the oral concentration time curve. R-mephenytoin plasma clearance was 33 ml/min. After the initial single dose of R-mephenytoin, this individual subject received daily doses of racemic mephenytoin, to achieve similar plasma levels of PEH and mephenytoin to those observed in figure 3. After the second oral i.v. dose of R-mephenytoin on day 11, the plasma concentration-time profile was similar to the first study; however, the terminal half-life had decreased to 8 hr. Plasma tritium activity decreased to a plateau indicating a similar volume of distribution of 1.08 liters/kg. Assuming that near steady state had been achieved, clearance could be calculated from the dose divided by the area under the plasma concentration time curve during the dosage interval. This indicated a 3-fold increase in systemic clearance to 106 ml/min.

Plasma protein binding. Plasma protein binding was independent of concentration over a range of 20 to 500 nmol/ml for both mephenytoin and PEH. The percentages of free drug for the S- and R-enantiomers of mephenytoin were 70.2 ± 2.3 and 63.8 ± 2.7%, respectively. The corresponding values for PEH were 81.0 ± 5.8 ard 71.5 ± 1.8%, respectively. None of the differences reached statistical significance. Thus, stereoselective drug metabolism cannot be attributed to stereospecific drug binding influencing availability of substrate for its metabolizing enzyme.

Discussion

Mephenytoin provides one of the most clearly defined examples in which stereospecific metabolism has a major influence on the availability of active drug moiety for therapeutic action. The implications of earlier observations based on differences in urinary excretion profile of metabolites of the enantiomers of mephenytoin after single dose administration (Küpfer *et al.*, 1981) have been supported in this study which has investigated the disposition of racemic mephenytoin and its metabolites in both plasma and urine during chronic drug administration. This study confirms that S-mephenytoin is rapidly metabolized by aromatic hydroxylation to 4-OH-M, a phenolic product which is rapidly and quantitatively eliminated in urine after glucuronidation. Furthermore, this stereospecificity is maintained during chronic drug administration. The rate of elimination of S-mephenytoin is sufficient to prevent drug accumulation when administered chronically on a daily basis. In contrast, the inability of the liver to hydroxylate R-mephenytoin in position 4 of the aromatic ring leads to the alternative metabolic pathway of oxidative demethylation to form PEH at a rate which is faster than its rate of renal elimination. As a consequence, R-PEH accumulates in the body. As racemic PEH is equipotent as an anticonvulsant in comparison to mephenytoin (Kupferberg and Yonekawa, 1975), it provides the major contribution to circulating hydantoin concentration during chronic therapy. Thus, under steady-state conditions only half of the racemic mephenytoin administered provides therapeutic efficacy.

The experimental design of the present study utilizes the approach of having different radiolabels for each enantiomer of mephenytoin and is based on the premise that each labeled enantiomer is handled similarly to the unlabeled enantiomer. This has been verified in previous studies (Küpfer *et al.*, 1981) in which an isotope effect was excluded in studies in which the ^{3}H: ^{14}C labeling pattern was reversed for the two enantiomers. This approach assumes that the time course of urinary excretion of radiolabeled metabolites, after the tracer dose, reflects the metabolic fate of that dose, irrespective of subsequent administration of unlabeled drug. It also implies that the metabolic fate of the second dose, given when an approximate steady-state situation had been achieved, would reflect the metabolic profile of drug after equilibration of complex processes such as nonlinear kinetics, drug accumulation and enzyme induction. In previous single dose studies, we observed stereoselective metabolism of mephenytoin in man (Küpfer *et al.*, 1981). This observation was confirmed as 95 ± 4% of the initial dose of S-mephenytoin was recovered in urine within the first 24 hr in the form of 4-OH-M and a small fraction in the second 24 hr (fig. 7). The rapid elimination of the S-enantiomer implies that this enantiomer should not accumulate after repeated doses. This prediction was substantiated by the constant daily excretion of unlabeled 4-OH-M during the study (fig. 5). The large recovery of [^{14}C]-S-4-OH-M, compared to [^{3}H]-R-4-OH-M, after both first and second administrations of radiolabeled drug confirmed that the extent of stereospecificity of aromatic hydroxylation was not altered by chronic therapy (table 1). With the long dosing interval relative to the rapid elimination of S-mephenytoin, any increase in drug metabolizing activity from that observed after the first dose would not be detected in the urinary excretion profile but would result in an increase in presystemic elimination so that the proportion of S-mephenytoin reaching the systemic circulation would be reduced. This might have contributed, at least in part, to the slight fall in plasma mephenytoin level over time (fig. 3).

The metabolic fate of the R-enantiomer of mephenytoin differs dramatically from that of the S-enantiomer. This difference is largely due to an inability of 4-hydroxylation of R-mephenytoin which persists during chronic therapy (table 1). As a consequence, R-mephenytoin is metabolized by other routes of metabolism. An attempt was made to specifically quantitate R-mephenytoin disposition in one subject. Differences in the unlabeled plasma concentration time profile in comparison to after racemic mephenytoin administration (fig. 9 and 2) could possibly have been due to stereoselective adsorption. Almost complete recovery of S-mephenytoin within 48 hr confirmed the complete absorption of the S-enantiomer. However, the delayed excretion of R-enantiomer made an assessment of its absorption more difficult. If man is similar to dog, then the volume of distribution of mephenytoin and PEH are similar (Küpfer and Bircher, 1979; Küpfer *et al.*, 1977). In this situation, the volume of distribution of ^{3}H should be the same as that of mephenytoin. The estimate of 1.46 liters/kg was similar to that obtained from estimating an apparent oral volume of distribution from unlabeled drug of 1.36 liters/kg. The similarity between these two indirectly derived estimates would be consistent with complete oral absorption of R-mephenytoin. Under this circumstance, it is justified to calculate an initial clearance of only 33 ml/min.

The metabolic fate of R-mephenytoin has not been fully elucidated; however, a major pathway is demethylation to form PEH, which is largely eliminated unchanged in urine with a low urinary clearance (Butler, 1952; Küpfer *et al.*, 1979). Approximately 10% of tritium in urine is present in the form of 4-hydroxy-PEH. However, it has not been established whether the conversion of PEH to 4-hyroxy-PEH is also stereoselective. The identity of the tritium recovered in urine not in the form

of these two metabolites is uncertain. It has been established that the tritium label of phenolic metabolites is acid labile and can be collected by distillation as tritium water after acid hydrolysis (Küpfer *et al.*, 1981). This strongly suggests that products of aromatic hydroxylation of mephenytoin and PEH to isomeric phenols, catechols or dihydrodioles can occur and that these products are present in urine.

Even though both R and S-enantiomers can form PEH, the relative differences in 4-hydroxylation result in the R-enantiomer contributing to a greater proportion of PEH after the first dose and this is accentuated during chronic therapy so that essentially all the PEH eliminated after the second radiolabeled dose was in the R configuration. In spite of the initial low clearance of R-mephenytoin and the fact that not all of it was converted to PEH, the rate of PEH production still exceeded the rate of urinary excretion. Consequently, the plasma PEH concentration gradually increased after repeated mephenytoin administration. It equalled the plasma mephenytoin level by the 3rd day, then increased progressively to reach a plateau by the 11th day, when there was a 10:1 ratio of PEH to mephenytoin (fig. 3). The 10:1 ratio between PEH and mephenytoin may well be an underestimate of the ratio dosing prolonged therapy, as the PEH half-life after discontinuation of mephenytoin therapy (fig. 4) suggests that true steady state would be achieved only after approximately 4 weeks so that further accumulation of PEH might be anticipated. The plasma concentration time profile is in contrast to the previous report by Troupin and co-workers (1979), who observed an immediate high rate of formation of PEH. It is possible tht differences between studies can be attributed to differences in the study populations. In the latter study, epileptic patients investigated had received prior anticonvulsant therapy with concomitant drug metabolizing enzyme induction; this contrasts to the normal drug-free subjects of this study.

The rate of accumulation of PEH is a complex process dependent on the ratio of the rate of production and the rate of elimination. Estimates of renal clearance of PEH varied between 2 and 5 ml/min and remained constant during this study, so that the rate of accumulation was largely determined by the rate of production. Three observations suggested that there was an increasing rate of PEH production over time. Firstly, in the subject who received R-mephenytoin alone on days 1 and 11, the plasma mephenytoin concentration time profile indicated an increase in systemic clearance from 33 ml/min on day 1 to 106 ml/min on day 11. Secondly, the urinary excretion of [^{3}H] PEH after the first dose of radiolabeled was low on the first day, then with successive doses of racemic mephenytoin being administered on subsequent days, the 24 hourly urine recovery increased to a peak by the 5th day before declining on subsequent days (fig. 8). This slow rise to a peak is suggestive of a greater rate of biotransformation on the 5th day and is supported by the greater amounts of [^{3}H]PEH excreted in urine in the days after the second radiolabel drug administration. A third more speculative observation was the plasma concentration time course of racemic mephenytoin within the first 24 hr of administration on days 1 and 11 (fig. 2). The initial rapid decline in plasma mephenytoin concentration can be interpreted as being due to the combined process of distribution of both S- and R-mephenytoin and the rapid elimination of S-mephenytoin, then the later plateau between 6 and 24 hr after the first dose could be due to the persistence of unmetabolized R-mephenytoin, together with delayed absorption of a proportion of the dose initially administered. On day 11 of treatment, the initial processes are the same as on day 1. However, a comparison of the change in plasma mephenytoin concentrations between 6 and 24 hr indicates a greater reduction than with the first dose. This would be consistent with induction in the metabolism of R-mephenytoin and could contribute to the decline in total plasma mephenytoin between days 3 and 14 (fig. 3). These observations suggest that chronic treatment with mephenytoin causes an increase in the rate of demethylation of R-mephenytoin in a similar manner to autoinduction observed with diphenylhydantoin. This would be consistent with the potent enzyme inducing properties of PEH in the rat (James *et al.*, 1981).

Although the production rate of PEH could not be determined, its subsequent rate of elimination after discontinuation of mephenytoin therapy was measured. The terminal half-life of PEH was 141 ± 19 hr (fig. 4); a value which is somewhat longer than previous reports (Troupin *et al.*, 1979). Thus, once an adequate circulating blood level has been achieved it should be possible to maintain adequate antiepileptic control with a once-weekly dose which could be given under supervision. This might provide a useful contribution to the management of patients who have difficulty with drug compliance.

In conclusion, the marked stereoselectivity of 4-hydroxylation of the phenyl ring of S-mephenytoin together with the relatively slow demethylation to R-PEH and even slower renal clearance results in a dramatic difference in the pharmacokinetic disposition of the S- and R-enantiomers of mephenytoin in man. As a consequence S-mephenytoin provides a negligible contribution to circulating hydantoins, whereas R-mephenytoin is converted to the pharmacologically active demethylated product R-PEH which is the major circulating hydantoin during chronic administration of the racemic drug.

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