Br. J. clin. Pharmac. (1981), **12**, 833–839

SERUM PHENYTOIN CONCENTRATION AND CLINICAL RESPONSE IN PATIENTS WITH EPILEPSY

D.J. GANNAWAY & G.E. MAWER

Department of Pharmacology, Materia Medica & Therapeutics,
University of Manchester Medical School, Oxford Road, Manchester M13 9PT

1 Patients with poorly controlled epilepsy were cautiously transferred from multiple drug therapy to treatment with phenytoin sodium alone. One patient suffered more severe seizures and the initial treatment was restarted. The remainder showed no deterioration.

2 The daily dose of phenytoin was then increased by a small increment at intervals of 2 or more months. The serum phenytoin concentration (total and free) was measured regularly and response was assessed by records of seizure frequency and tests of speech, handwriting, short-term memory and coordination.

3 Patients ($n = 11$) with partial seizures showed no consistent improvement with increased phenytoin concentration within the range 15 mg/l (60 μmol/l) to the individual threshold for intoxication, $\geq$ 35 mg/l (140 μmol/l). Patients ($n = 4$) with generalized seizures however were consistently improved at higher concentrations.

4 Tolerance to phenytoin varied, the threshold for symptomatic intoxication ranging from 35–60 mg/l (140–240 μmol/l) total and 2.7–5.2 mg/l (10.8–20.8 μmol/l) free. Ataxia was the commonest symptom and in some cases this was manifest by worsening of performance on the test of coordination (pursuit rotor). Even at lower phenytoin concentrations the patients performed less well on this test than control subjects. Other tests of performance showed no evidence of impairment at higher phenytoin concentrations.

5 The same daily dose of phenytoin tended to give higher serum drug concentrations after intoxication than before.

Introduction

It has been common practice to prescribe antiepileptic drugs in combination (Guelen, Van der Kleijn & Woodstra, 1975) with the expectation of more effective seizure control or equivalent control with milder side effects than could be obtained from larger doses of a single drug. The practice has however been challenged because the evidence for greater efficacy is weak (Shorvon & Reynolds, 1977) and combinations increase the opportunities for adverse drug effects (Reynolds, 1975).

The emphasis has now shifted to finding the optimum amount of one drug for a particular patient by progressive dosage adjustment and serial measurement of serum drug concentration. The usually accepted target for phenytoin is a concentration of 10–20 mg/l (40–80 μmol/l; Bucthal, Svensmark & Schiller, 1960; Kutt *et al.*, 1964; Lund, 1974). Signs of intoxication are often not detectable however until the concentration approaches 40 mg/l (160 μmol/l) (Brooker & Darcey, 1973).

When seizure control remains unsatisfactory despite a serum phenytoin concentration of 10–20 mg/l (40–80 μmol/l), two options can therefore be considered, to add other drugs or to make further dosage increase. We have taken a small group of epileptic patients with poor seizure control despite trial of the first option, cautiously removed the other drugs and tried the second. The objective was to obtain better seizure control by exploring the serum concentrations between the usually recommended range and the individual threshold level at which toxicity became just detectable.

Methods

Patients

Eighteen patients with poorly controlled partial complex, generalized tonic/clonic or focal motor seizures were studied (Table 1). All were able to comprehend the study objectives and give informed consent. All lived independently in the community and twelve were in full-time employment or managing a home

0306–5251/81/120833–07 $01.00

and family. Fourteen were initially receiving multiple antiepileptic drug therapy (phenytoin 13, carbamazepine 4, primidone 4, sodium valproate 3, sulthiame 2, clonazepam 2 and phenobarbitone 1). Three were receiving phenytoin alone. During Phase 1 patients were gradually transferred to treatment with phenytoin alone in sufficient dosage to produce initial serum concentrations in excess of 15 mg/l (Gannaway & Mawer, 1981). During Phase 2 the daily dose of phenytoin was increased in 25 mg steps every few months, until seizures were completely suppressed or clinical features of mild intoxication were detected. In the latter event daily dosage was immediately reduced by one step. At each dosage level seizure frequency was recorded and performance measured in tests of speech, handwriting, short-term memory and coordination (pursuit-rotor). Venous blood was taken for determination of total and free serum phenytoin concentrations. To ensure that data were not collected whilst serum concentrations were changing substantially no observations were made during the first 10 days after a dosage change. A minimum of three attendances at intervals of one month were made at each dosage level.

Daily dosage was usually divided into 2 (morning and evening) although patients on doses up to 400 mg daily were encouraged to take it as a single dose. The time of blood sampling in relation to dosage was not standardised but tended to be consistent within each patient. The difference between peak and trough serum phenytoin concentration is known to be small and to be virtually uninfluenced by dosage interval up to 24 h (Buchanan *et al.*, 1972).

Phenytoin determination

Total phenytoin serum concentration was determined by a modified method of MacGee (1970), using a Perkin-Elmer F30 gas chromtograph, 3% OV 225, on Gas Chrom Q 80–100 mesh and 5-(*p*-methylphenyl)-5-phenylhydantoin as internal standard. Phenytoin protein binding was determined by a radiolabelled ultracentrifugation technique. Two ml aliquots of serum together with 10 μl of 5,5-diphenyl-[4-^{14}C]-hydantoin in ethanol (10 μCi/ml) were transferred to polycarbonate tubes. The contents were agitated and 100 μl aliquots transferred to vials containing 10 ml of scintillant (Packard 299). The tubes were capped and centrifuged for 15 h at 250,000 *g* and 37°C. After centrifugation 100 μl aliquots of the supernatant were transferred to vials containing 10 ml of scintillant. All vials were counted in a liquid scintillation counter. The proportion of unbound phenytoin (U) is given by:

$$U = \frac{\text{d/min after centrifugation}}{\text{d/min before centrifugation}} \times 100\%$$

Free phenytoin concentrations were calculated by multiplying the unbound proportion by the total concentration determined by gas chromatography.

Seizure frequency

Every patient recorded in a diary the epileptic events experienced. At each attendance these were discussed, classified and recorded on a standard sheet. The total number of seizures in each consecutive 7 day period was termed the seizure frequency. This was not a 'blind' assessment although the current serum phenytoin concentration was not known until later. Most of the patients had received detailed diagnostic investigation in the Departments of Neurology or Medicine in the Manchester Royal Infirmary. The classification of seizure type (Table 1) was based on this and on the history obtained from the patient or family. The attendances were long enough (about 1 h) and numerous enough (mean 18 range 11–24) to ensure that the authors witnessed a seizure in most patients.

Speech

At each attendance the patient's voice was recorded whilst reading a short passage. Speech was assessed 'blind' for dysarthria by a final year speech therapy student who listened to the patient's tape at a later date. She assigned each recording to a position on a four point scale–A normal, B mild, C moderate and D severe dysarthria.

Table 1 Details of the patients studied

Main seizure type	*Number of patients*	*Age (years) Mean (range)*	*M*	*F*	*Duration of epilepsy (years) Mean*	*(range)*	*Seizure frequency (per week) Mean*	*(range)*
Partial complex	11	32.8 (18–47)	6	5	15.2	(3–29)	6.5	(0.5–35)
Generalized tonic/clonic	5	31.6 (16–54)	4	1	18.0	(4–29)	1.5	(0.5–4)
Focal motor	2	39.5 (30–49)	1	1	13.5	(3–24)	0.5	(0.3–0.5)

Handwriting

A test of handwriting was used as it has been reported that the size of writing increases in the presence of a CNS depressant drug (Legge, Steinberg & Summerfield, 1964). The test consisted of the patient writing the same passage at each attendant. The performance score used was the sum of the lengths of ten 'key words' contained in the passage.

Short-term memory

A test of short-term memory was used as it has been reported that high phenytoin concentrations may cause mental impairment (Kutt *et al.*, 1964). The apparatus consisted of a tape recorder and a pre-recorded tape containing ten groups of 15 words. After hearing each group of words the patient was asked to repeat as many as he could remember in 1 min. The mean of the number of correct words in each group gave the score used on this test.

Motor coordination

Coordination was assessed using a pursuit-rotor consisting of a variable speed turntable with an eccentric metal target close to the perimeter (Electronic developments, Hampton, Middlesex). When a probe electrode touched the target a timer operated. At each of four rotor speeds the patient was asked to keep the probe on the target for as long as possible during a run of 1 min. To allow for practice and fatigue effects the rotor speeds were duplicated in reverse order, so that a complete test consisted of determinations at 30, 40, 60, 80, 80, 60, 40 and 30 rev/min. Mean time on target was plotted against speed of turntable. The score used was the area under the curve (AUC) of the least squares linear regression line extrapolated to intercepts on both x and y axes. This score gives a measure of performance over the entire speed range. Impairment of performance is manifest as decreased AUC. One problem in attempting to discover a relationship between pursuit rotor score and phenytoin concentration was the presence of progressive improvement with practice (Figure 1). A mathematical technique was devised to correct for this trend. Each patient's pursuit rotor data was fitted by an iterative computer technique to the equation:

$$y_n = y_\infty - (y_\infty - y_o)\, e^{-kn}$$

where y_n is the score at the nth attempt
y_∞ is the theoretical maximum score achievable by the patient
y_o is the theoretical score before any practice
k is a constant describing the rate of improvement
and n is the number of tests performed.

For each observed value y_n the computer produced a calculated value. Residuals were determined by subtracting observed from calculated values. These residuals were plotted against total phenytoin concentration and tested for regression. A *t*-test on the correlation coefficient was used to determine whether there was a significant correlation between residuals and phenytoin concentration.

Tests of statistical significance

Seizure frequencies within groups of patients at high and low phenytoin concentrations were compared using paired tests (Wilcoxan or *t* as applicable). Frequencies at different phenytoin concentrations within individuals were compared using non-parametric methods. Both χ^2 and Kruskal-Wallis tests (Siegel, 1956) were used. No single test was satisfactory for every patient, as there was large individual variation in the range and distribution of seizure frequencies.

Results

Transfer from multiple drug therapy to treatment with phenytoin alone (Phase 1) was achieved without

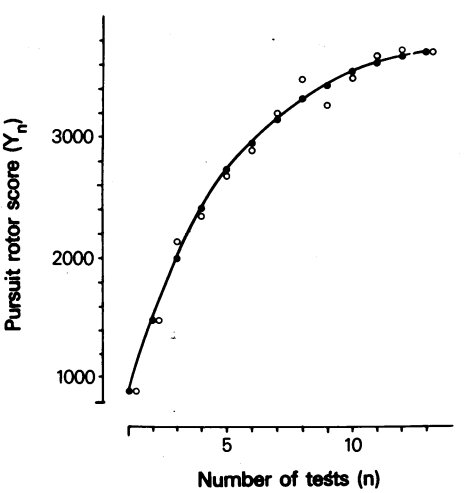

Figure 1 Improvement of pursuit rotor score with repetition in one patient (IG). The experimental scores y_n (○) are plotted against the number n of tests performed. The computer fitted scores y^1_n (●) are based on the arbitrary assumption that the rate of improvement declines in proportion to the differences between y^1_n and the ultimate score y_∞. The improvement is described by an exponential rate constant k (tests^{-1}).

In three patients only there was a significant ($P < 0.05$) correlation between impairment of pursuit rotor performance (residual, $y^1_n - y_n$) and serum phenytoin concentration.

significant loss of seizure control except in one patient (BF) who suffered two unusually severe seizures at the final stage of primidone withdrawal. The results of this phase of the study have been published separately (Gannaway & Mawer, 1981). The above patient and four others were withdrawn from Phase 2 of the study for the reasons outlined in Table 2.

Table 2 Patients withdrawn from the study

Patient	*Main seizure type*	*Reason for withdrawal*
BF	PC	Generalized tonic/clonic seizures following transference to single drug therapy
AH	PC	Erratic phenytoin serum levels
AJ	FM	Seizure-free at phenytoin serum levels below 15 mg/l
CS	G	Erratic phenytoin serum levels
KW	PC	Defaulted

Seizure frequency

The thirteen remaining patients each provided seizure frequency data within two non-overlapping ranges of serum phenytoin concentration. In every case the mean of the higher range was greater than 20 mg/l (80 μmol/l).

The relationship between the mean frequency of partial seizures and the mean serum phenytoin concentration is shown in Figure 2. With the exception of MP (focal motor) and PS (markedly attenuated variants of tonic/clonic seizures) all were complex in type. In no case was total suppression of seizures obtained. Six patients showed a decrease in seizure frequency at the higher concentration but one (KS) showed no change and four showed an increase. In the group as a whole there was no significant difference betwen the frequencies of partial seizures at higher and lower phenytoin concentrations. When individuals were considered in isolation only one (MO) showed a significant ($P < 0.05$) change (reduction) in seizure frequency at the higher phenytoin concentration.

The relationship between the mean frequency of generalized tonic/clonic seizures and the mean serum phenytoin concentration is shown in Figure 3. Two patients had their generalized seizures totally suppressed at the higher phenytoin concentration and all four showed a decrease in seizure frequency. This was statistically significant in the group as a whole ($P < 0.05$) and in the individual case of TH ($P < 0.0005$).

Toxicity

Eight patients experienced symptoms of phenytoin intoxication (Table 3). Ataxia was the commonest

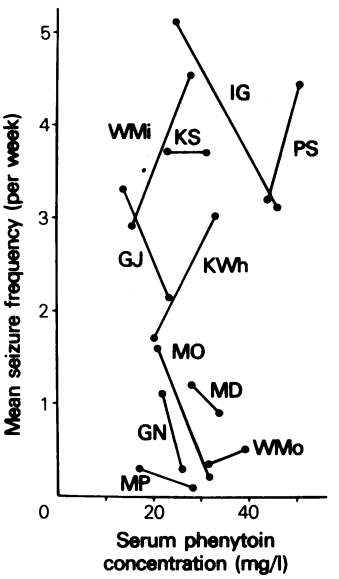

Figure 2 Influence of serum phenytoin concentration on seizure frequency (per week) in 11 patients with partial seizures. Each point represents the mean of 6 to 23 weeks of seizure record and 2 to 10 measurements of drug concentration. Within each patient the concentration ranges corresponding with the two means did not overlap. There was no consistent evidence of more effective suppression of seizures at higher phenytoin concentration.

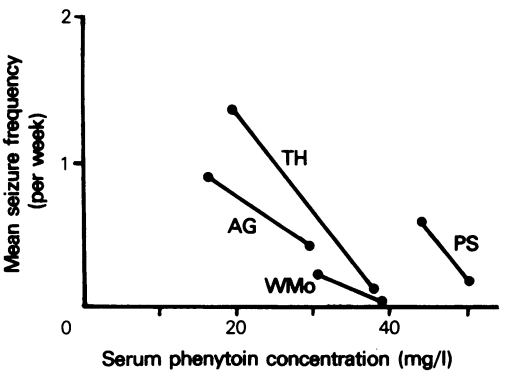

Figure 3 Influence of serum phenytoin concentration on seizure frequency (per week) in four patients with generalized tonic/clonic seizures. Each point represents the mean of 7 to 25 weeks of seizure record and 4 to 12 measurements of drug concentration. Within each patient the concentration ranges corresponding with the two means did not overlap. There was consistent evidence of more effective suppression of seizures at higher phenytoin concentration.

Table 3 Serum phenytoin concentrations at which patients first reported symptoms of intoxication

Patient	*Phenytoin concentration (mg/l)*		*Symptoms*
	Total	*Free*	
PS	60	5.2	AT, DI, DY
WMo	45	4.2	PA
MD	39	3.5	AT
TH	38	3.3	AT, DI
CS	38	3.2	AT
KWh	35	3.2	AT
MO	36	2.9	AT
KS	39	2.7	AT, DI, DY

AT ataxia, DI diplopia, DY dysarthria, PA paraesthesiae (possibly coincidental)

symptom and diplopia was reported more frequently than had been expected. Only two patients (or their relatives) reported dysarthria. It is not certain whether patient WMo was intoxicated. He complained of 'pins and needles' in his feet. Since peripheral neuropathy has been attributed to phenytoin (Reynolds, 1975) this possibly coincidental symptom was taken as a contraindication to further dosage increase. The serum phenytoin concentrations at which symptoms were first reported are shown in Table 3. No patient reported symptoms at concentrations below 35 mg/l (140 μmol/l) total or 2.7 mg/l (10.8 μmol/l) free. One (PS) did not develop symptoms until concentrations of 60 mg/l (240 μmol/l) total and 5.2 mg/l (20.8 μmol/l) free.

The assessment of toxicity did not include nystagmus as observer error was found to be large and early attempts at reproducible measurement had proved disappointing.

A lag phenomenon was observed amongst those patients in whom the dose of phenytoin was progressively increased to the point of intoxication and then decreased. There was a tendency for serum concentrations on the same daily dose to be higher after intoxication than before. Table 4 shows the mean serum concentration at a fixed dose before and after intoxication. The highest phenytoin concentration measured in each of the patients is also included. The differences between serum levels before and after intoxication appeared possibly to be directly related to the highest observed phenytoin concentration.

Performance tests

Of the four performance tests only the pursuit rotor appeared to be a potential tool for detecting phenytoin intoxication. Even this test failed to identify signs of intoxication at the time of testing. In retrospect however three patients showed a significant ($P < 0.05$) negative correlation betwen pursuit rotor performance and phenytoin concentration after correction for the practice effect (**Methods**, Figure 1).

The performance of the patients as a group on the pursuit rotor was compared with that of a group of healthy volunteers. These were matched with the patients for age and sex but were receiving no long-term drug treatment. In Table 5 the two groups are compared on the basis of theoretical maximum performance (y_∞) and the rate of improvement with practice (k). The performance of the patients was significantly poorer ($P < 0.01$, Mann-Whitney U-test) by both criteria and seven individual patients had significantly lower ($P < 0.05$) maximum performance scores.

Performance in the tests of speech, handwriting and short-term memory was not detectably impaired at the time of symptomatic phenytoin intoxication. Similarly no relationship between performance and phenytoin concentration was found. No significant difference in short-term memory was found between the patients and the healthy volunteers.

Discussion

The first phase of the study was relatively trouble-free except for the one patient who developed more severe seizures after primidone withdrawal. Amongst the remainder there was no evidence that seizure control was impaired by transfer to treatment with phenytoin alone (Gannaway & Mawer, 1981).

Table 4 Serum concentrations on a fixed dose of phenytoin before and after intoxication (ranked according to the highest measured phenytoin concentration)

Patient	*Dose (mg/day)*	*Serum phenytoin concentration (mg/l)*		
		Mean before intoxication	*Mean after intoxication*	*Highest measured*
PS	475	46	55	65
WMo	400	31	36	45
MD	475	27	30	39
KS	500	31	30	39
KWh	550	21	25	35

Table 5 Summary of pursuit rotor performance in epileptic patients and age matched control subjects

Control subjects	*k (number of tests^{-1})***	*Y_∞***
CC	0.34	4730
MC	0.31	4182
RC	0.26	4728
AG	0.32	4252
JT	0.34	4881
JV	0.49	4054
Mean	0.34	4471
Patients		
MD	0.17*	2989*
AG	0.25	3751*
IG	0.24	3873
TH	0.19*	2797*
GJ	0.31	2596*
WMi	0.29	3669*
WMo	0.26	2804*
GN	0.30	4040
MO	0.26	4174
MP	0.39	4271
KS	0.18*	3508*
CS	0.22	4208
KWh	0.24	4134
Mean	0.25	3601

* Significantly different from healthy control group ($P < 0.05$)

** Calculated as described in **Methods** and illustrated in **Figure 1**.

It proved possible to explore phenytoin serum concentrations up to 35 mg/l (140 μmol/l) without symptoms of intoxication but there were problems with dosage adjustment. Increments in daily dose of 25 mg (Mawer *et al.*, 1974) although suitable for most patients occasionally produced an excessive rise in steady state concentration. In one case for example the mean concentration increased from 19 to 38 mg/l (67 to 152 μmol/l) when daily dose was increased from 400 to 425 mg. To produce an intermediate concentration (28 mg/l; 112 μmol/l) it was necessary to prescribe 400 and 425 mg on alternate days.

At each dosage level the serum concentration varied between visits. In two cases (Table 2) large variation prevented evaluation of concentration/response relationships and in one or two others it was necessary to ignore data collected from an isolated period during which concentrations had been erratic. Despite these difficulties we were able to collect from 13 patients seizure and performance data at two distinct concentration levels between 15 mg/l (60 μmol/l) and the individual threshold for intoxication; observations were made for 2–6 months at each level and the concentration limits within each individual did not overlap.

There was no consistent evidence (Figure 2) that patients with partial seizures obtained any additional benefit from phenytoin concentrations above 20 mg/l (80 μmol/l). The group was divided almost equally between those who improved and those who failed to improve or deteriorated. It is suspected that uncontrolled changes in work or family circumstances and seasonal factors had a greater influence on seizure frequency than did the systematic increase in phenytoin concentration.

The response of the patients with generalized tonic/clonic seizures (Figure 3) by contrast was consistent and encouraging. Three of these had suffered severe disruption of their lives by frequent grand mal attacks despite a variety of drug combinations. In this context it is probably justifiable to deliberately maintain relatively high phenytoin concentrations whilst continuing to monitor for possible chronic toxicity (Reynolds, 1975). In one patient (PS) the reduced frequency of generalized tonic/clonic seizures at high serum phenytoin concentration (Figure 3) was associated with an increased frequency of the attenuated variants (Figure 2). No general evidence was found however to support the widely held belief that high phenytoin concentrations cause an increase in seizure frequency (Lascelles, Kocen & Reynolds, 1970).

The lowest threshold serum concentration of free phenytoin for symptomatic intoxication, 2.7 mg/l (10.8 μmol/l) (Table 3) was the same as that reported by Booker & Darcey, 1973. There was little individual variation (90.7–92.9%) in the extent of serum protein binding confirming the results of Lund, Berlin & Lunde (1972). Abnormal protein binding was therefore not the reason for the remarkable tolerance of one patient (PS) to high serum concentrations of phenytoin. Patients perceived symptoms of intoxication before impairment was detected by any formal test. The most sensitive of the tests, the pursuit rotor revealed impairment at higher phenytoin concentrations but not in every case of subjective intoxication and only after correction for the practice improvement effect. The remainder of the tests failed to show any within-patient differences between performance at higher and lower phenytoin concentrations.

The most impressive result from the tests was the poor performance of the patients relative to the controls on the pursuit rotor (Table 5). Within the patient group there was no relationship between the length of history or the apparent severity of the epileptic seizures and the pursuit rotor score. It is possible therefore that phenytoin may be responsible for detectable impairment of coordination even at serum concentrations well below the level at which the patient perceives himself to be ataxic.

The observation that the same daily dose of phenytoin can give higher serum concentrations after

intoxication has been made previously (personal communication from Professor A. Richens) but has not apparently been published. One tenable hypothesis may be that phenytoin has accumulated in a slow compartment, possibly adipose tissue, and that the daily oral dose is effectively supplemented over several months by slow release from such a depot.

Dr R.G. Lascelles and his colleagues in the Department of Neurology, Royal Infirmary, Manchester gave valuable advice at the planning stage and referred several of the patients. Mrs D.B. Byers Brown (Department of Audiology and Education of the Deaf, University of Manchester) enabled two senior speech therapy students Mrs Linda Talbot (1978/1979) and Mrs Patricia Waterman (1979/1980) to participate. They willingly undertook the laborious task of scanning a large number of tape recordings for evidence of dysarthria. D.J. Gannaway was supported by a research grant from Warner-Lambert (UK) Ltd who also generously met the cost of reagents and materials.

References

BOOKER, H.E. & DARCEY, B. (1973). Serum concentrations of free diphenylhydantoin and their relationship to clinical intoxication. *Epilepsia*, **14**, 177–184.

BUCHANAN, R.A., KINKEL, A.W., GOULETT, R.J. & SMITH, T.C. (1972). Metabolism of diphenylhydantoin following once-daily administration. *Neurology*, **22**, 126–130.

BUCHTHAL, F., SVENSMARK, O. & SCHILLER, P.J. (1960). Clinical and electroencephalographic correlations with serum levels of diphenylhydantoin. *Arch. Neurol.*, **2**, 624–630.

GANNAWAY, D.J. & MAWER, G.E. (1981). Transfer from multiple to single antiepileptic drug therapy. *Lancet*, **i**, 217.

GUELEN, P.J.M., VAN DER KLEIJN, E. & WOODSTRA, U. (1975). Statistical analysis of pharmacokinetic parameters in epileptic patients chronically treated with antiepileptic drugs. In *Clinical pharmacology of antiepileptic drugs*, eds. Schneider, H., Janz, D., Gardner-Thorpe, C., Meinardi, H. & Sherwin, A.L. p. 2. Berlin: Springer.

KUTT, H., WINTERS, W., KOKENGE, R. & McDOWELL, F. (1964). Diphenylhydantoin metabolism, blood levels and toxicity. *Arch. Neurol.*, **11**, 642–648.

LASCELLES, P.T., KOCEN, R.S. & REYNOLDS, E.H. (1970). The distribution of phenytoin levels in epileptic patients. *J. Neurol. Neurosurg. Psychiat.*, **33**, 501–505.

LEGGE, D., STEINBERG, H. & SUMMERFIELD, A. (1964). Simple measures of handwriting as indices of drug effects. *Perceptual and motor skills*, **18**, 549–558.

LUND, L. (1974). Anticonvulsant effect of diphenylhydantoin relative to plasma levels. *Arch. Neurol.*, **31**, 289–294.

LUND, L., BERLIN, A. & LUNDE, P.K.M. (1972). Plasma protein binding of diphenylhydantoin in patients with epilepsy. *Clin. Pharmac. Ther.*, **13**, 196–200.

MacGEE, J, (1970). Rapid determination of diphenylhydantoin in blood plasma by gas-liquid chromatography. *Analyt. Chem.*, **42**, 421–422.

MAWER, G.W., MULLEN. P.W., RODGERS, M., ROBINS, A.J. & LUCAS, S.B. (1974). Phenytoin dose adjustment in epileptic patients. *Br. J. clin. Pharmac.*, **1**, 163–168.

REYNOLDS, E.H. (1975). Chronic antiepileptic toxicity: A review. *Epilepsia*, **16**, 319–352.

SHORVON, S.D. & REYNOLDS, E.H. (1977). Unnecessary polypharmacy for epilepsy. *Br. med. J.*, **1**, 1635–1637.

SIEGEL, S. (1956). *Nonparametric statistics for the behavioural sciences*. New York: McGraw-Hill.

(Received April 3, 1981)