CARBAMAZEPINE AND ITS METABOLITES IN EPILEPTIC PATIENTS

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To my parents to whom belong my respect and gratitude.

C O N T E N T S

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SUMMARY

Carbamazepine is a drug which is now widely used for the treatment of both generalised epilepsy (tonic-clonic seizures) and partial epilepsy (with simple or complex symptomatology).

This study was undertaken in an attempt to assess the role of the metabolites of carbamazepine, viz. 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine, with regard to their therapeutic efficacy and the occurrence of side effects of the parent drug. It was also designed to seek a possible explanation as to why certain patients with optimal levels of carbamazepine in plasma fail to respond to therapy.

A total of 23 epileptic patients (11 females and 12 males) suffering from either generalised (tonic-clonic) seizures or partial complex seizures took part in the study. The patients were divided into two groups according to their seizure frequency:

Responders - those patients who had no seizures in the month prior to entry into the study (12 patients).

Non-Responders - those patients who had a minimum of one seizure a week in the month prior to entry into the study (11 patients).

Carbamazepine and its metabolites were monitored between 8 a.m. and 6 p.m. by taking blood samples at two hourly intervals. Cerebrospinal fluid (CSF) was also obtained from seven patients in the non-responder group. The drug and its

metabolites were assayed simultaneously by the thin-layer chromatographic (TLC) method of Hundt and Clark (1975).

Six of the 23 epileptic patients complained of side effects: nausea and headaches were the most frequently mentioned complaint. Statistical analysis showed, however, that there was no significant difference in the peak levels of carbamazepine and metabolites in patients both with or without side effects. Therefore it was not possible to define a threshold level of the drug above which side effects were likely to occur. Also no definite conclusion could be reached as to whether the metabolites play a role in the manifestation of side effects.

The area under the curve (AUC) is a measure of the overall plasma concentration of carbamazepine and metabolites (present between 8 a.m. and 6 p.m.) in the individual patients of the two groups. There was no significant difference in the AUC of carbamazepine between responders and non-responders. However, the AUC of the dihydroxy and epoxy metabolites was significantly higher in the non-responders ($P < 0.002$ and $P < 0.02$ respectively). Moreover in the CSF sampes of the non-responders, the mean (±SD) ratio of the dihydroxy metabolite to the parent drug was as high as 1.17 (±0,36).

The results show a clear association between high levels of metabolites and poor response to carbamazepine therapy. Furthermore it would seem that either both metabolites are inactive or that if the epoxy metabolite is active as in the rat

(Frigerio and Morselli 1975), any likely therapeutic effect is counter-acted by the relatively large concentration of inactive dihydroxy metabolite (Schmutz et al 1979). Moreover, it may follow that non-response to carbamazepine - despite optimal levels of the drug in plasma - may be due to competition by inactive dihydroxy metabolite for the site (s) of action of the parent drug in the brain. Research stratergies which might be used to test this hypothesis have been proposed.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

CHAPTER ONE

1.1. Introduction

Carbamazepine was first synthesised in the laboratory of J.R. Geigy AG (Basel, Switzerland) in 1952. In the early 1960's it underwent extensive tests in animals (Theobald and Kunz 1963; Lorge 1963; Jongmans 1964) and was found to possess powerful anticonvulsant properties. The drug came into clinical use in 1963, for the treatment of epilepsy, in Great Britain and Europe. In 1968 it came into use in America for the relief of the pain of trigeminal neuralgia. It was only in 1974 that the marketing of carbamazepine for use in the treatment of epilepsy was approved by the Food and Drug Administration (FDA) of the U.S. Department of Health, Education and Welfare.

In epilepsy, carbamazepine is now used quite extensively in the treatment of generalised (tonic-clonic) seizures and partial seizures (with elementary or complex symptomatology).

In its two-dimensional structure, carbamazepine is quite dissimilar to the older anticonvulsants diphenylhydantoin and phenobarbitone (Fig. 1). However, in making a structural comparison between two compounds, one has to view the three-dimensional structure as well (Fig. 2). On doing so, one notes that the structural differences between carbamazepine and diphenylhydantoin may be more apparent than real (Julien and Hollister 1975).

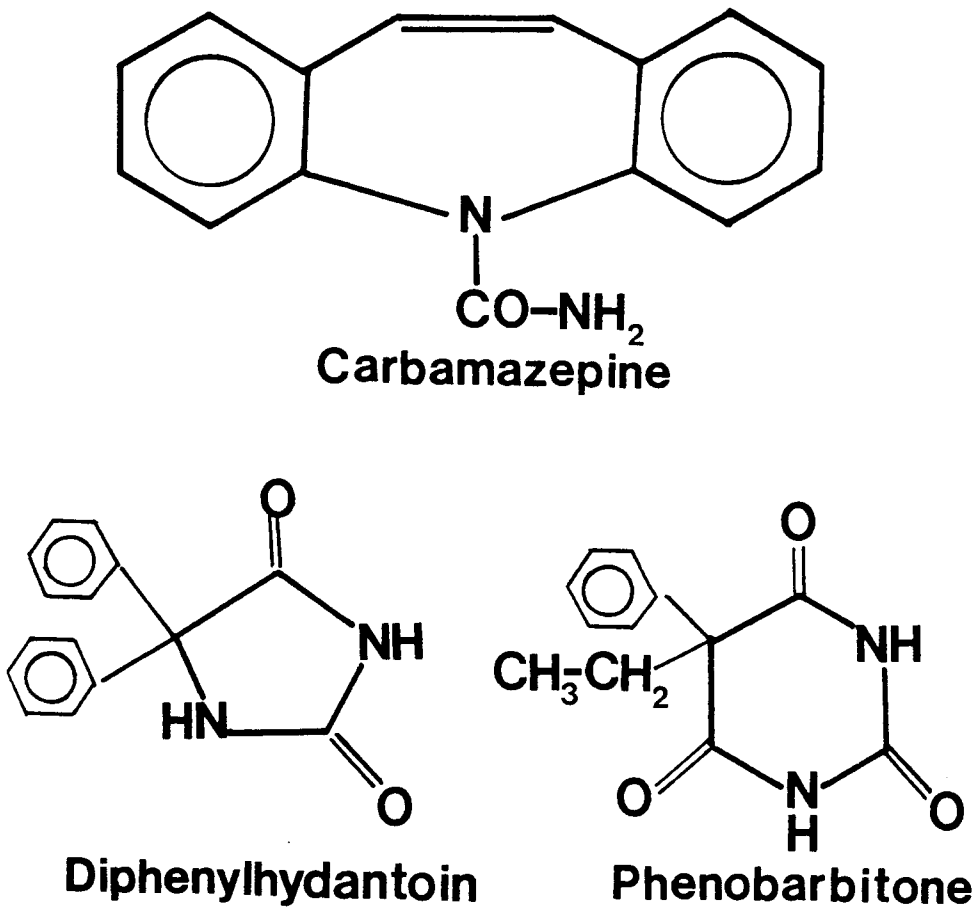


Fig. 1 Chemical structures of carbamazepine, diphenylhydantoin, and phenobarbitone.

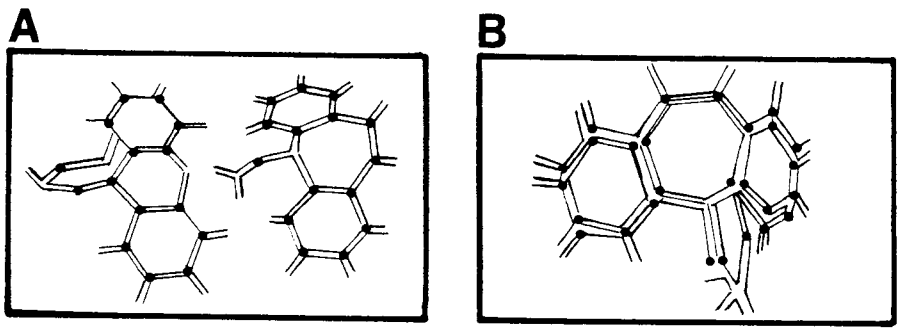


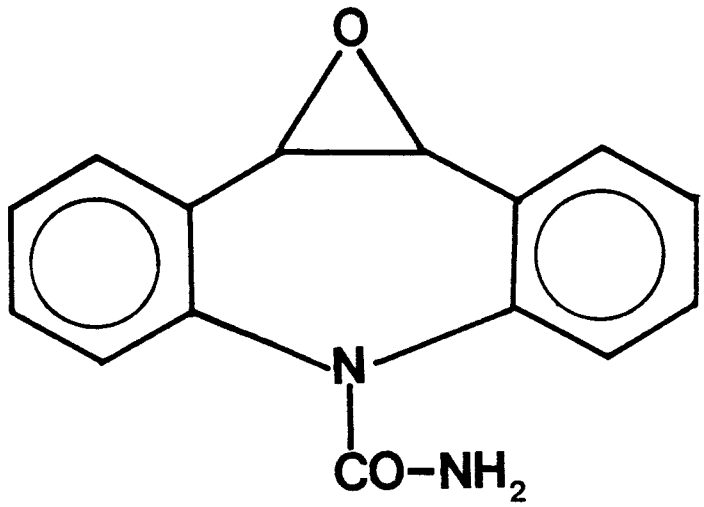
Fig. 2 A. Molecular models of diphenylhydantoin and carbamazepine.
B. Superimposed models to illustrate three-dimensional similarity. (Adapted from Julien and Hollister 1975).

Since the two structures can be superimposed, it seems likely that the two drugs should have similar pharmacologic properties; in fact both are powerful anticonvulsants, both have the ability to alleviate intractable pain (e.g. in trigeminal neuralgia) and both depress digitalis-induced ventricular arrhythmias. However, carbamazepine differs from diphenylhydantoin in one important aspect: the phenyl rings of carbamazepine are rigidly fixed by the ethylidene bridge and this means that unlike diphenylhydantoin it has no behavioural depressant properties which diphenylhydantoin shares with the barbiturates.

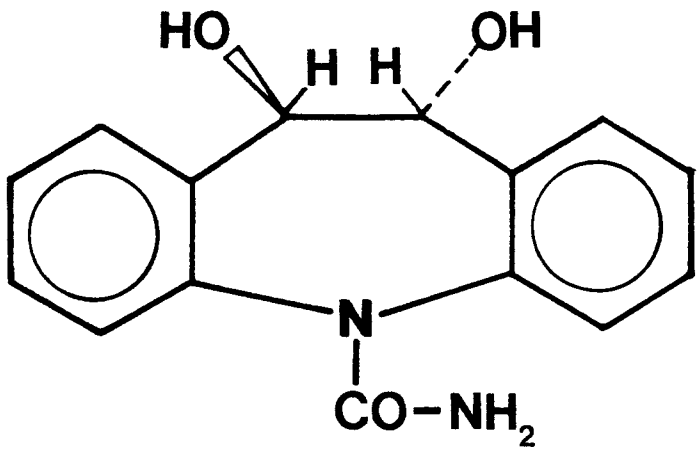
This important property of carbamazepine together with its ability to depress convulsive activity has made it a valuable drug in the change from polypharmacy to monotherapy in the treatment of epilepsy.

Carbamazepine is a drug which is almost completely metabolised in the body. The two major metabolites that are present in plasma are: 10,11-epoxy-carbamazepine (EPOXY-CBZ) and 10,11-dihydro-10,11-dihydroxy-carbamazepine (DI-OH-CBZ) (Fig. 3).

Unlike the majority of epoxides, the epoxide metabolite of carbamazepine is comparatively stable and seems no more toxic than carbamazepine itself (Glatt et al 1975). EPOXY-CBZ has been shown to possess definite anticonvulsive properties in rats (Frigerio and Morselli 1975), but it is still not certain whether this metabolite plays a role in the overall anticonvulsant effect of carbamazepine in man.



10,11-Epoxy-Carbamazepine



10,11-Dihydro-10,11-Dihydroxy-Carbamazepine

Fig. 3 Chemical structures of 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine.

DI-OH-CBZ is formed by the enzymatic cleavage of the epoxide metabolite. It has no anticonvulsant activity (Schmutz et al 1979). However, although it is a polar metabolite it persists in plasma in measurable quantities 15-24 hours after a dose and is even present in cerebrospinal fluid. This metabolite may therefore be in part responsible for the side-effects and toxicity sometimes encountered with carbamazepine therapy.

1.2. Aims of the Study

This project was designed to investigate:

(a) To what extent the metabolites of carbamazepine play a role in the therapeutic efficacy and side-effects of the drug.

(b) To discover a possible explanation as to why certain patients with optimal levels of the drug in plasma do not respond to therapy.

There are very few reports in the literature in which the metabolites of carbamazepine have been monitored during therapy with the drug. In most of these studies no definite conclusion was reached as to the part played by the metabolites in the therapeutic efficacy and side-effects of carbamazepine. There are no reports on studies carried out on non-responders to carbamazepine.

1.3. Review of the Literature

1.3.1. Anticonvulsant Efficacy of carbamazepine

The first controlled trial to evaluate the efficacy of carbamazepine as a new anticonvulsant was reported by Bird et al (1966). The study was carried out double blind for a period of 18 months in 45 mentally sub-normal in-patients suffering from generalised (tonic-clonic) seizures. Twenty-four of these patients were started on carbamazepine and gradually weaned away from the other anticonvulsants until they were on carbamazepine monotherapy. The remaining 21 patients acted as the control group and consisted of patients taking phenobarbitone, diphenylhydantoin and primidone either alone or in combination. After the trial period there was a 26% improvement in the seizure frequency in patients taking carbamazepine alone and 16% improvement in the control group. The results are not statistically significant; however, they suggest that carbamazepine has an equivalent anticonvulsant action to the older drugs. Side-effects were rarely reported; they included anorexia in one patient in the carbamazepine and control groups and loss of balance (1-3 days) in 2 patients in the carbamazepine group and one patient in the control group. No haematological abnormalities were detected in any of the patients taking part in the trial.

In America, although carbamazepine came into clinical use in 1974 for the treatment of epilepsy, a trial was reported by Livingstone et al (1967) to evaluate its potential use. Eighty-seven patients suffering from various forms of epileptic seizures took part in the trial. They

had all been refractory to treatment with one or more of the standard epileptic drugs, 20 of the patients became completely seizure-free, 37 failed to respond to therapy, 30 showed marked improvement. Carbamazepine was found to be particularly useful in the control of psychomotor (temporal lobe) epilepsy. In addition to its anticonvulsant properties carbamazepine also produced a marked improvement in behaviour and personality in some patients. The trial also showed that carbamazepine could produce various forms of side-effects during its use. The most prominent of these was diplopia. In most cases this reaction occurred in the early stages of treatment and disappeared spontaneously. Other untoward reactions encountered were transient blurred vision, drowsiness, ataxia and leucopenia.

In South Africa, carbamazepine came into clinical use in the late sixties and a long-term clinical trial was reported by Wulfsohn (1972), to determine its value as an anticonvulsant and psychotropic drug. The patient population was 29 white adult males suffering from generalised (tonic-clonic) seizures who had proved refractory to treatment with two or more anticonvulsant drugs. In 26 out of the 29 patients a marked improvement in the seizure frequency was reported over a period of 2½ years on addition of carbamazepine to the treatment regimen. In 2 patients seizures remained unchanged and one deteriorated. Psychotropic improvement (mood, sociability, work capacity and personality) was equally striking: 27 of the 29 patients improved, often dramatically, and none deteriorated. Side-effects encountered included mild

initial nausea and dizziness in two cases. The author points out that this could either have been due to good toleration or to the fact that the group as a whole was mentally disturbed.

After carbamazepine was initially introduced, its full acceptance as a new anticonvulsant was delayed because several reports about the occurrence of aplastic anaemia with its use appeared in the literature (Spillane 1964; Donaldson and Graham 1965; Dyer 1966; Saleh and Medes de Leon 1968; Fellows 1969). It is noteworthy that since 1969 there have been no reported cases of aplastic anaemia and it remains questionable as to whether or not carbamazepine was responsible for those anaemias.

1.3.1.1. Studies of Efficacy in Children

The first trial in children was reported by Scheffner and Schiefer (1972). Seventy-four children aged between 1 and 15 years were treated for period of 2 to 7 years with carbamazepine in combination with the standard anticonvulsant drugs. Two-thirds of the children had generalised (tonic-clonic) seizures and the rest partial seizures (focal and psychomotor). Eighty-nine percent of the children became seizure-free one to three years after the start of carbamazepine therapy.

The daily dose necessary to achieve freedom from seizures depended on body weight. Children weighing 10-20 kg required a mean of 24 mg/kg, or roughly twice the dose (11 mg/kg) necessary for children weighing 50-60 kg.

No side-effects were encountered during therapy. The only significant changes in the electroencephalogram (EEG) during treatment with carbamazepine were a decrease in focal spikes in children 9-12 years old, and a diminished incidence of focal slowing in all children.

In Israel a study was carried out to investigate the effectiveness of carbamazepine as the sole drug in the focal epilepsy of childhood, comparing it with diphenylhydantoin and the barbiturates (Lerman and Kivity-Ephraim 1974).

In all 40 patients were studied in the age range 4-11 years. Twelve patients had carbamazepine as their first and only drug whereas in the others various other drugs and drug combinations had previously been administered.

After the trial period of 26 months, 32 patients were seizure-free from the initial dosage of carbamazepine. In six, seizures ceased when the dose was increased (the maximum was 600 mg/day) and the remaining two had neglected to take their medication. In 80% of the children with behavioural problems, behaviour improved when carbamazepine was given.

Only one child developed a rash on carbamazepine and no other side-effects were observed. EEG improvement occurred in 25% of the patients but in the remaining cases the focus persisted although the patients remained seizure-free.

Numerous other reports dealing with the effectiveness of carbamazepine in the treatment of epilepsy in childhood have since been published (Grant 1972; Rett 1973; Gamstorp 1976).

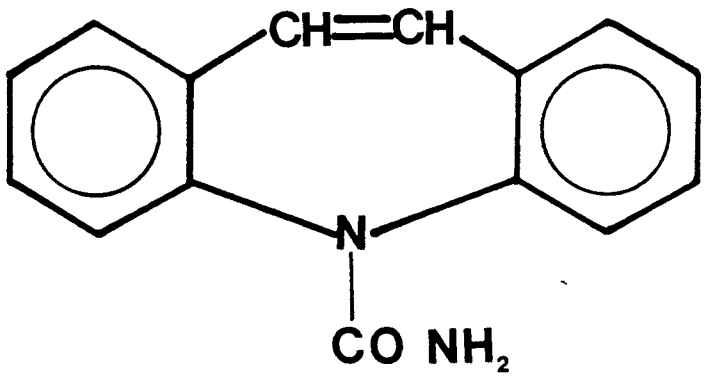
1.3.2. Psychotropic effect of carbamazepine

Carbamazepine is a drug which is closely related in its two-dimensional structure to the psychotherapeutic agent imipramine (Fig. 4).

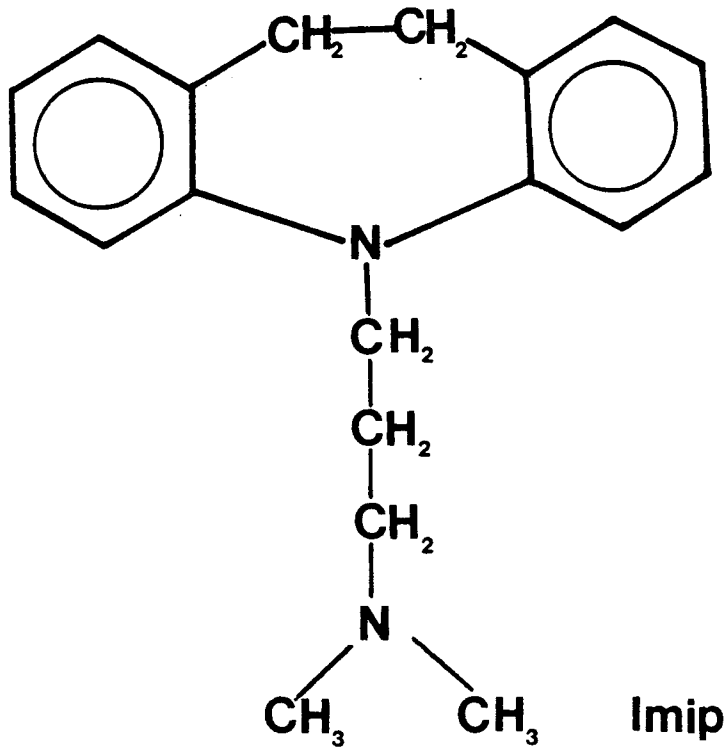
The early clinical trials (Livingstone et al 1966; Pryse-Philips and Jeavons 1970; Wulfsohn 1972) reported that patients using carbamazepine experienced a beneficial effect on behaviour (diminuition of aggressiveness, improved co-operation in the wards, etc.) and in performance (increase in alertness, measured abilities, etc.). These observations were attributed to the tricyclic structure of carbamazepine.

However, there could have been two possible explanations for this positive psychotropic effect of carbamazepine: firstly, in all these studies carbamazepine had been substituted for the more intoxicating anticonvulsants, for example, phenobarbitone and diphenylhydantoin. Secondly, the improvement in seizure frequency by the addition of carbamazepine to the treatment regimen could well have accounted for the improvement in behaviour and performance.

Rett et al (1976) reported on a long-term pharmaco-psychological investigation carried out to establish whether carbamazepine did indeed have a psychotropic effect. The patient population consisted of 30 children and adolescents (12 girls and 18 boys) suffering from generalised (tonic-clonic) seizures. They were divided into three groups:



Cabamazepine



Imipramine

Fig. 4: Chemical structures of carbamazepine and imipramine.

Group A: Patients who had previously been treated with phenobarbitone, phenytoin and primidone in various combinations before receiving CBZ monotherapy.

Group B: Patients who had not received any anticonvulsants for at least 6 months before entry into the study.

Group C: Patients taking primidone, mephenytoin and phenytoin either alone or in combination.

The group A patients had their first psychological examination while they were still receiving their previous treatment. They were then transferred over a period of 1 to 2 weeks to carbamazepine monotherapy. The second psychological investigation was undertaken 11-12 months later.

The psychological examination involved the following tests:

(a) Hamburg-Wechsler Intelligence scale for children: Verbal IQ, Practical IQ and Overall IQ.
(b) Walther test: Psychomotor function.
(c) Rorschach test: Activity and psychic manifestations.

At the end of the 12 month trial period no change could be demonstrated in the group A patients. No significant change was demonstrable in the group C patients although there was a trend towards improvement in a number of tests. The group B patients showed a significant improvement in the Walther test and this was the only evidence that could be found for a possible psychotropic effect for carbamazepine.

No other studies have been reported since on the possible beneficial psychotropic effect of carbamazepine in epileptic patients. Therefore this question is still a controversial subject.

1.3.3. EEG Studies

Patients on phenobarbitone and diphenylhydantoin often show a marked improvement in their electroencephalographic (EEG) tracings when they respond to therapy (Buchthal and Svensmark 1960-1).

A double-blind study was therefore undertaken to compare the effect of carbamazepine and placebo on the EEG and fit frequency of a group of long-stay epileptic patients in a psychiatric hospital (Pryse-Phillips and Jeavons 1970).

All patients had epilepsy of partial (temporal lobe) or generalised type and were allocated randomly to receive either carbamazepine or an identical placebo. The first EEG record, which included the response to hyperventilation and photic stimulation, was made before the administration of carbamazepine or placebo, the second after the patients had received 600 mg daily for a month and the third a month after the drug had been discontinued.

At the end of the trial period, no EEG improvement was seen among the patients taking carbamazepine. In fact in 20% of the patients the EEG was worse (i.e. a greater increase in focal spiking) while they were receiving the drug, and the EEG abnormality disappeared after the drug was discontinued.

No correlation could be found between the EEG findings and the frequency of seizures.

Ferrer-Vidal et al (1976) investigated whether there was a correlation between EEG background activity and serum levels of carbamazepine and phenobarbitone. Fifty patients took part in the study and EEG background activity was evaluated on the same days as the serum level determination of carbamazepine and phenobarbitone.

It was found that there was no correlation between the slowing down of background activity and the serum levels of carbamazepine. On the other hand, high serum levels of phenobarbitone did go hand in hand with lower EEG background activity, while serum phenobarb levels of less than 30 µg/ml were associated with normal EEG background activity of 8,5 Hz.

In a long-term study carried out over a period of 12 months, Monaco et al (1979) analysed the EEG tracings in 20 epileptic patients suffering from either generalised or partial seizures. The patients received carbamazepine either alone or in combination with phenobarbitone and/or phenytoin and/or clonazepam. EEG was performed twice monthly.

The findings suggested that the majority of the tracings do not worsen, but nor do they improve. The majority of the patients displaying freedom from seizures had good carbamazepine plasma levels and an irregular EEG tracing.

The authors therefore concluded that EEG was of little value in assessing the clinical picture of patients on carbamazepine.

1.3.4. Serum levels

In the present day management of epilepsy, serum level determination of anticonvulsants play an important role; the physician can ensure that the patient is compliant or that he is receiving a high enough dose to be therapeutic but not toxic.

Our current knowledge of the therapeutic serum concentrations of carbamazepine comes from a variety of studies and clinical trials.

Frey and Yryana (1970) were the first people to report a therapeutic concentration for carbamazepine. They measured carbamazepine in serum by the method of Hermann and Geigy (1965), in 23 adult epileptic patients receiving 400-1 000 mg/day of the drug. Serum titres of 1-13 µg/ml (average 5 µg/ml) were obtained. About 60% of the serum concentrations were from 4-6 µg/ml, 20% from 7-10 µg/ml and 8% from 1-3 µg/ml. The highest single value was 13 µg/ml. All the above levels were associated with seizure control. The authors point out that no cases of carbamazepine intoxication were included in their study.

Parsonage (1972) determined carbamazepine concentrations by the method of Gardner-Thorpe et al (1972) in 48 children and adults. He concluded that there was no relationship between serum concentrations and degree of seizure control. Dosage range was 100 to 1 400 mg/day and serum concentrations ranged from non-detectable to 11,8 µg/ml. Only one patient was receiving carbamazepine as sole drug (400 mg/day) and the level was 8,8 µg/ml, but the seizures were not controlled (as cited by Cereghino 1975).

Meinardi (1972) studied the correlation between serum carbamazepine levels and clinical control and side-effects in six epileptic patients. He predicted that 50% of all patients on carbamazepine will suffer side-effects such as diplopia, ataxia, headaches and dizziness at serum levels of 8,5-10 µg/ml whereas nystagmus could occur at much lower serum levels (1,5 µg/ml) in an appreciable number of patients.

Johannessen and Strandjord (1973) reported carbamazepine concentrations in serum and CSF of 19 adult epileptic patients (8 women and 11 men), and their relation with dosage in mg/kg body weight. Serum and CSF samples were taken before the morning dose and carbamazepine was determined by a modified Hermann and Geigy method (1965). The mean concentrations of carbamazepine in serum and CSF were 7,6 and 1,7 µg/ml, respectively, without any significant differences between men and women. Because of the low protein content of CSF, the concentration in CSF can be taken as the therapeutically active unbound fraction of the drug. This was found to be 22 ± 5% of the total serum concentration. The ratio between dosage in mg/kg and concentration of carbamazepine in serum showed marked individual differences and, therefore, the authors advised that dosage should be adjusted according to the serum level in each patient.

Two independent studies carried out in America, to evaluate the efficacy of carbamazepine for epilepsy, included serum level determination as part of their clinical trial protocols. Cereghino et al (1974) studied 36 institutionalised adults receiving 1 200 mg of carbamazepine daily for a period of 21 days. Serum concentrations ranged up to 13,7 µg/ml,

with half the values ranging from 5,8-9 µg/ml. Troupin et al (1974) studied carbamazepine in 12 patients, aged 19-54 years. Mean oral dosage was 16,8±4 mg/kg, and mean serum concentration was 10,7 µg/ml. In both studies, a wide variety of side-effects was encountered, especially on initiation of therapy, but not severe enough to discontinue the drug. No mention is made as to the serum levels at which these side-effects occurred.

Schneider (1975) investigated whether there was a correlation between early morning ('fasting') serum levels of carbamazepine and the degree of seizure control, and also at what serum levels side-effects occurred. (Samples were taken 3 hours after the morning dose for this latter correlation.) Of the patients studied, 94 were hospitalised and 75 institutionalised, i.e. had been under observation for many years. Carbamazepine was estimated by the method of Hermann (1965). Good seizure control was obtained with mean serum levels of 4,6±1,3 µg/ml in institutionalised and 6,5±3,0 µg/ml in hospitalised patients. Carbamazepine serum levels related to the first appearance of side-effects such as drowsiness, ataxia and diplopia, averaged 11,6±4 µg/ml. The threshold level above which side-effects were likely to occur was estimated to be 8,9 µg/ml in serum.

Eichelbaum et al (1976) reported plasma levels of carbamazepine and one of its main metabolites, carbamazepine-10,11-epoxide, during treatment of epilepsy. Twenty-five patients suffering from complex partial seizures with or without generalization of the seizures took part in the study. The mean dose of carbamazepine was 12,5±3,3 mg/kg body weight.

Blood samples were collected before the morning dose and carbamazepine and its epoxide metabolite were assayed by a liquid chromatography method of Eichelbaum and Bertilsson (1974). The mean concentration of carbamazepine and its epoxide metabolite were 5,4±2,5 µg/ml and 1,1±0,42 µg/ml, respectively. In two patients simultaneous measurements in CSF and plasma were carried out. The ratio between the concentration of carbamazepine in CSF to plasma was of the order of 0,22 as compared to 0,45 for the epoxide. The authors point out that the larger unbound fraction of the epoxide suggests that this metabolite could contribute to the anticonvulsant effect of carbamazepine if it is as active in man as in rats (Frigerio and Morselli 1975).

Monaco et al (1976) monitored carbamazepine levels weekly over a period of nine weeks in 20 epileptic patients unresponsive to treatment. The patients received carbamazepine either alone or in combination with phenobarbitone and/or phenytoin. Carbamazepine was administered either twice or three times daily according to the individual needs, in doses of 5-24 mg/kg body weight. Blood samples were collected twice daily at 8 a.m. and 6 p.m. and the concentration of carbamazepine and its epoxide determined by a gas chromatography method of Morselli et al (1975). No attempt was made to alter phenytoin or phenobarbitone levels; emphasis was on achieving carbamazepine levels of 4-10 µg/ml. Within 2-3 weeks of monitoring, there was a remarkable drop in the seizure frequency with carbamazepine plasma levels in the desired range. There was no relationship between the epoxide and carbamazepine concentrations. This study showed a definite

improvement in the seizure frequency could be achieved if carbamazepine levels were kept in the therapeutic range defined by the authors.

Dam et al (1977) reported on a study carried out to evaluate whether carbamazepine-10,11-epoxide had an independent anti-epileptic activity in man. The patient population included 132 epileptic out-patients treated for 1 to 8 years with carbamazepine alone or in combination with phenytoin, phenobarbitone or both. One hundred and eighteen patients had generalised (tonic-clonic) seizures and 57 had partial seizures with complex symptomatology. Carbamazepine and its epoxide were determined by a TLC method described by Christianssen (1973). A significantly higher level of epoxide was found in patients on combined treatment compared with patients on carbamazepine alone. However, no significant difference in epoxide levels could be demonstrated between the patients whose seizures were well controlled and those who were non-responders to carbamazepine. Hence, it appeared that the epoxide had no additional anti-convulsant properties in man.

In most studies dealing with serum levels and seizure control, so far described, carbamazepine was used in combination with other anticonvulsant drugs. Callaghan et al (1978) carried out a study in which carbamazepine was used as a single drug in the treatment of epilepsy. Thirty-two patients with a variety of seizures took part in the study. Blood samples were collected at intervals which varied from 1 to 8 hours after the last dose of carbamazepine, and serum levels were measured by gas liquid chromatography according to the method of Rogers et al (1973). Thirteen patients became completely seizure-free and

10 had a greater than 50% reduction in seizures. The range of levels associated with: (i) no seizures was 1,2-8,1 µg/ml; (ii) greater than 50% reduction in seizures 5,7-12,6 µg/ml; and (iii) in non-responders 7,4-20,5 µg/ml. The study therefore failed to define a therapeutic range for carbamazepine as a wide range of serum levels was associated with freedom from seizures. This discrepancy could have arisen as a result of the wide variation in sampling times.

Monaco et al (1979) reported on a long-term study on carbamazepine in which the relationship between drug plasma levels, EEG and adverse reactions were studied over a 12 month monitoring period. Carbamazepine was used as monotherapy or in combination with phenobarbitone and/or phenytoin and/or clonazepam. The subjects taking part in the study included 16 adults and 4 children, suffering from generalised or partial seizures. Blood samples were collected at 8 a.m. and 6 p.m. on one day per month, and plasma levels of carbamazepine and the epoxide were determined according to the method of Morselli et al (1973). The mean seizure frequency decreased from 6,6 seizures per year to 1,2 seizures per year on inclusion of carbamazepine in the study. The therapeutic level of carbamazepine was found to be 7-9 µg/ml. The epoxide metabolite was found to be present in those patients whose carbamazepine plasma levels exceeded 4-5 µg/ml. However, no correlation could be found between the type of EEG pattern and the plasma levels of carbamazepine. In this study it was shown that the efficacy of carbamazepine was prolonged and constant particularly in patients on monotherapy and the authors point out that the treatment of epilepsy should

as far as possible begin with one drug alone - carbamazepine.

Hoppener et al (1980) carried out a study to determine whether there was a correlation between the daily fluctuations in carbamazepine serum levels and intermittent side-effects. Sixty-two patients using carbamazepine either alone or in combination with other anticonvulsants and experiencing side-effects such as drowsiness, diplopia and headaches, took part in the study. Carbamazepine was administered three times a day and blood samples were collected at 2 hour intervals starting just before the morning dose at 8 a.m. and ending at 6 p.m. Serum levels were determined by gas chromatography using the method described by Cramers et al (1976). Each patient showed a more or less prominent increase in serum levels 2 hours after intake. In the afternoon a maximum was reached, often attended by side-effects. After changing the mode of administration (reducing the lunchtime dose) and the serum level remaining below 8 μg/ml, the side-effects disappeared. None of the patients showed an increase in seizure frequency after this change.

Johannessen and Strandjord (1980) reported a therapeutic serum concentration of 3,1-12,1 μg/ml for carbamazepine. This was derived from a study in which carbamazepine was used as a sole drug in the treatment of epilepsy in 62 patients. Serum concentrations of carbamazepine and its epoxy metabolite were determined by enzyme immunoassay and gas chromatography, respectively, in fasting morning blood specimens. Of the 62 patients, 49 became seizure-free while 13 remained non-responders to the drug even with optimal serum levels of carbamazepine and the epoxide. No reasons were given for this discrepancy.

1.3.5. Toxicity of carbamazepine

Anticonvulsant drugs are administered in physiologically active doses to epileptic patients over prolonged periods of time and patients may even take daily doses throughout most of their lifetime. Such prolonged therapy with a rather potent pharmacological agent is likely to affect not only the central nervous system but normal physiologic functions as well. The toxicologic effect produced in patients taking an anticonvulsant drug, which in this case is carbamazepine, can be divided into different groups:

(1) Acute - resulting from drug overdosage.
(2) Idiosyncratic drug reactions.
(3) Drug interactions.
(4) Teratogenic.

1.3.5.1. Acute toxicity

The therapeutic range for carbamazepine that has been put forward by the various clinical trials can be averaged out to be something like 4-12 μg/ml. This is of course subject to interindividual variations. Usually acute toxicity results when the upper limit of the therapeutic range is exceeded as a result of taking an overdosage of the drug whether iatrogenic, accidental or purposeful. In most cases, carbamazepine intoxication presents itself by clinical signs and symptoms of central nervous system dysfunction. The cerebello-vestibular and the pyramidal extrapyramidal systems are the ones that are greatly affected. Higher cortical function and reticulocortical activating system are only mildly disrupted (Wilder 1976).

Cerebello-vestibular dysfunction results in nystagmus, ataxia and incoordination. Extrapyramidal effects are unmasked when toxicity becomes severe, the results of which are dystonic posturing, choreiform and athetoid movements and signs of asterixis.

Since the introduction of carbamazepine in 1962, for the treatment of epilepsy and trigeminal neuralgia, there have been relatively few cases reported of acute toxicity due to the drug.

De Zeeuw (1979) reported on a severe case of carbamazepine intoxication in which 16 g of carbamazepine was ingested in an apparent suicide attempt. Plasma levels of the drug and its epoxy metabolite were monitored by HPLC. On admission, the patient was in a semicomatose state but arousable. After two days of treatment (gastric lavage and aspiration), the patient lapsed into deep coma and the plasma levels of carbamazepine and the epoxide were found to have increased from 29-44 μg/ml and 14-22 μg/ml, respectively. Rapid elimination of the drug then followed with complete recovery within 4 days. The authors point out that the anticholinergic and central depressant properties of carbamazepine could have brought about a marked decrease in intestinal motility resulting in a protracted absorption. When motility was restored, rapid absorption took place resulting in increased levels of the drug and its metabolite. They suggest that in cases of carbamazepine intoxication gastric lavage and aspiration should be carried out up to a period of 3-4 days after ingestion.

Only two other reports have appeared in the literature giving details of the fate of the drug during carbamazepine intoxication (Gruska et al (1969) and Gulzow et al (1975)). However, the assay procedure used in these papers was based on the formation of 9-methylacridine and this therefore gave the sum of carbamazepine and carbamazepine epoxide in the blood.

Jacome (1979) reported on carbamazepine-induced dystonia in four patients suffering from intractable seizures. Three of the patients developed transient dystonia of the axial muscles, and one of the hands, after carbamazepine was added as anticonvulsant therapy. All the patients were taking more than 1 000 mg/day when the symptoms appeared. Serum levels of carbamazepine were not determined.

Crosley et al (1979), in their paper on dystonia associated with carbamazepine administration in three brain-damaged children, point out that carbamazepine is a structural analogue of the phenothiazines and tricyclic antidepressant drugs. This could therefore mean that the production of dystonia and choreoathetoid movements is the result of carbamazepine having a direct antagonistic role at the dopamine receptors.

A report was published in 1976, by Chadwick et al, on asterixis and cerebellar syndrome due to carbamazepine intoxication. The case involved a patient taking 1 200 mg/day of carbamazepine to control the pain of trigeminal neuralgia. On admission she had nystagmus, moderate gait ataxia and asterixis of her outstretched hands. The serum level at the time was 41 μmol/l (9,7 μg/ml). The dose of carbamazepine

was gradually reduced to 800 mg/day. The asterixis improved but was still present. Serum carbamazepine level had fallen to 6 µmol/l (1,7 µg/ml). On withdrawal of carbamazepine asterixis disappeared.

In 1975, Troupin and Ojemann reported on a new syndrome - paradoxical intoxication. They defined this as an increase in the seizure frequency as the blood level of the anti-convulsant rises. This phenomenon is rare and can occur when using high doses of an anticonvulsant with resulting high serum levels to obtain seizure control. One case is described where carbamazepine was used as sole drug and an increase in seizure frequency (from 0 per month to 6 per month) occurred when the blood level of the patient exceeded 20 µg/ml. Reduction in dose and blood level resulted in a decrease in seizures. The authors make the point in their paper that this should not discourage the gradual increase in the dose in order to achieve a successful balance between seizure control and the usual medication side-effects.

As far as toxicity is concerned, carbamazepine is a relatively innocuous drug compared to the barbiturates and phenytoin. No deaths have so far been reported with carbamazepine overdosage.

1.3.5.2. Idiosyncratic drug reactions

Idiosyncratic or adverse reactions to a drug refer to those reactions that are generally unpredictable when therapy is started. They are not related to dose and may occur at various stages in the course of treatment. Idiosyncratic reactions are rare but nevertheless important as many are serious, even potentially fatal.

The adverse reactions to carbamazepine will be classified according to the bodily systems affected:

Organ or Organ System	Adverse Reaction	Mechanism	References
Skin	Exfoliative dermatitis Urticaria	Allergic	Houwezijl et al (1978) Ramsay (1967)
Haematopoietic	Leukopenia Aplastic anaemia Thrombocytopenia	Allergic or direct toxicity	Gerber et al (1979) Rutman (1978) Dyer et al (1966) Pisciotta (1975) Pearce (1968)
Autoimmune	Lupus erythematosus reaction Thyroiditis	Allergic or direct toxicity	Takigawa (1976) Rootwelt (1978)
Liver and Kidney	Hepatitis Jaundice Water intoxication		Krudsen and Jensen (1979) Tur-Kaspa and Levo (1978) Perucca (1978)
GIT Tract	Nausea and vomiting		
Cardiovascular	Bradycardia		Herzberg (1978)
Central Nervous System	Psychoses, e.g. hallucinations		Berger (1971)

1.3.5.3. Drug interactions

It is only in recent years that the dangers of drug interactions have been realised and despite the present enthusiasm for monotherapy in the treatment of epilepsy, polypharmacy is still widely used. When anticonvulsant drugs or drugs for other disease states are added, the patient's reaction may not simply be caused by the added effects of the additional drug but by the interaction of the drug combination within the individual.

The drug interactions that have so far been reported for carbamazepine (CBZ) are listed below:

Interfering drug	Clinical significance	References
Phenytoin Phenobarbitone Primidone	Reduced therapeutic efficacy of CBZ, usually of little clinical significance because of the added effect of the interfering drug.	Christiansen and Dam (1973) Cereghino et al (1975) Johannessen et al (1975) Schneider (1975) Dam et al (1975) Rane et al (1975)
Warfarin	Decreased serum warfarin and impaired hypothrombonemic response. Reduction in warfarin half-life.	Hansen et al (1971)
Propoxyphene Triacetyloleandomycin	Rise in serum levels of CBZ which may lead to toxicity.	Dam and Christiansen (1977) Dravet et al (1977)
Lignocaine Quinidine	Therapeutic efficacy reduced by CBZ.	Perucca and Richens (1979)
Doxycycline	Half-life reduced by CBZ therefore possibility of reduced anti-bacterial effect.	Penttilla et al (1974)
Steroid contraceptives	Reduced contraceptive efficacy	Stockley (1976) Hempel and Klinger (1976)

Interfering drug	Clinical significance	References
Monoamine-oxidase inhibitors (MAOI)	No clinical reports of interactions have been reported but on theoretical grounds it may be dangerous to administer CBZ with MAOI	
Lithium	Inhibition of lithium-induced polyuria	Macallum (1980) Perucca and Richens (1980) Ghose (1980)
Cimetidine	Inhibition of CBZ metabolism - may lead to toxicity	Tellerman-Toppet et al (1981)

1.3.5.4. Teratogenicity

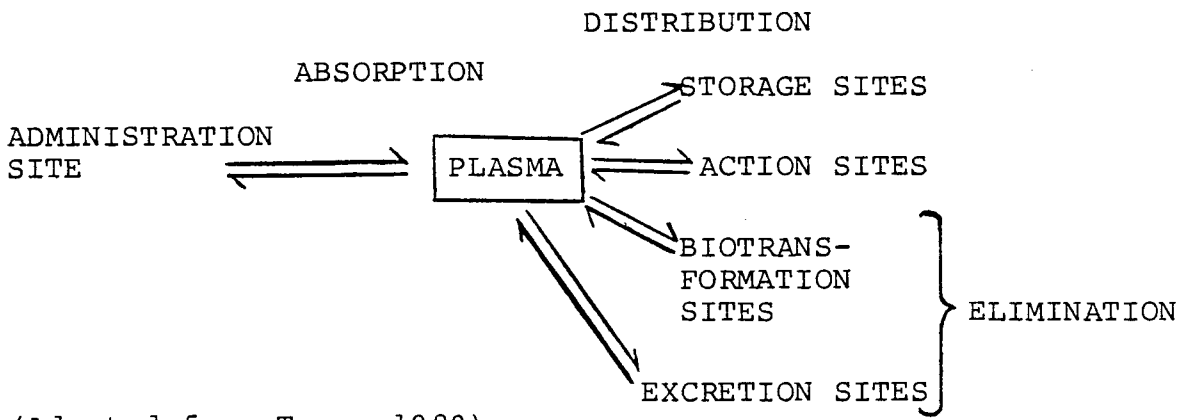
Pregnancy complicates the problem of seizure control since a seizure during gestation could have disastrous effects on the developing foetus because of the consequent anoxia. The use of anti-epileptic drugs prophylactically is therefore extremely important. However, it has been shown that drugs like phenytoin, phenobarbitone, primidone and trimethadione are unequivocally implicated in human dysmorphogenesis (Meadow 1970; Meyers 1973; Annegers et al 1974; Yoshimbi et al 1980). In the case of carbamazepine, Starreveld-Zimmerman et al (1973) reported that there was a reduced incidence of malformations in mothers who took this drug alone, or in combination with other anticonvulsants during pregnancy. Hicks (1979), on the other hand, described one instance of defective organogenesis in the stillborn offspring of a mother who took carbamazepine only in the first 6-8 weeks of pregnancy.

It can therefore be seen that the literature neither confirms nor denies the teratogenicity of carbamazepine and more studies are required to indicate a clear preference between no treatment and carbamazepine, with carefully monitored serum levels, for epilepsy in pregnancy.

1.3.6. Pharmacokinetics of carbamazepine

Nowadays it is considered essential for rational therapy that the full pharmacokinetic profile of a drug should be known. This is because it has become evident that the extent to which a drug exerts its therapeutic, and even its toxic effects, depends on its biological availability and disposition in the body.

The processes involved in getting a drug to its site of action and terminating its particular pharmacological effect can be summarized in the following schematic diagram:



(Adapted from Tyrer 1980)

1.3.6.1. Absorption and Bioavailability

Carbamazepine is a drug which, from its physical and chemical properties, is classified as a neutral lipophilic compound. The absolute bioavailability of carbamazepine in man has not been determined, because a preparation suitable for intravenous injection is not available. However, a study carried out by Faigle and Feldmann (1975), in which 400 mg of 14 C-labelled carbamazepine was administered to two healthy

volunteers, found that the oral bioavailability of the drug is more than 70%. This was based on the recovery of radio-labelled carbamazepine in urine and faeces.

Levy et al (1975) found that the bioavailability of carbamazepine could be increased by administering the tablets with meals because dissolution rather than disintegration is the crucial step for absorption. Solubility of the drug is increased by the bile secreted following a meal.

The time to reach peak plasma concentration, T_{max}, has been shown to vary from something like 6 to 24 hours, after single doses (Hvidberg and Dam 1976; Faigle et al 1976). However, it must be pointed out that the T_{max} is the time at which the elimination of a drug comes to exceed its amount of absorption. It is therefore a measure of both the absorption rate and elimination rate of the drug. A drug does not necessarily have to reach its T_{max} value in order to exert its particular pharmacological effect. A better parameter which can give an idea of how fast a drug is absorbed is the absorption half-life, $T_{\frac{1}{2}abs}$, and for carbamazepine this is 1,72 hours (Levy et al 1975).

1.3.6.2. Plasma Protein Binding

The plasma protein binding of carbamazepine has been measured by direct measurements (equilibrium dialysis and ultrafiltration) and by simultaneous drug concentrations in CSF and saliva, relative to drug concentration in whole plasma. There is rather good agreement between the various studies which gave protein binding of carbamazepine as 70 to 80%

(Johannessen and Strandjord 1972; Meinardi 1972; Di Salle et al 1974; Johannessen et al 1976).

Hooper et al (1975) found that patients with hepatic disease had a significantly lower protein binding than patients without hepatic disease, but the difference was not large enough to be of practical importance. Renal disease had no influence on the extent of binding of carbamazepine to plasma proteins.

Morselli et al (1975) and Rawlins et al (1975) showed that drugs like phenytoin and phenobarbitone, which are very often administered concomitantly with carbamazepine, had no influence on the protein binding of the latter.

Schneider and Berenguer (1975) made simultaneous measurements of CSF and plasma concentrations of the two major metabolites of carbamazepine, viz. 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine. A mean (±SD) CSF/plasma ratio of 48,9±9,6% was found for the epoxy metabolite and 47±9,3% for the dihydroxy metabolite. Moreover, it was found that complex concurrent administration of other medications had no displacing effect on the extent of protein binding of the epoxy metabolite but in the case of the dihydroxy metabolite not only did the total amount increase but also its free fraction.

1.3.6.3. Distribution

Because carbamazepine is neutral, it is a fairly lipophilic compound and passes easily through membranes in the body. After single oral doses of carbamazepine to volunteers or patients, the approximate volume of distribution has been

found to vary between 0,79 to 1,86 ℓ/kg body weight. These figures were calculated assuming complete bioavailability of the drug (Bertilsson 1978).

1.3.6.4. Biotransformation

Biotransformation is important for both the intensity and the duration of pharmacological effects since the rate limiting step of drug removal from the body is metabolism not excretion. As with other drugs, carbamazepine is reacted in the liver to form more polar metabolites which, being water soluble and able to be conjugated, are more readily excreted in bile and urine.

The main pathways of metabolism of carbamazepine that have been identified are shown in fig. 5.

Taking the total urinary radioactivity as 100%, the following approximate percentages are attributable to the different pathways:

1.	Epoxidation of the 10,11 double-bond of the azepine ring	40%
2.	Hydroxylation of the six-membered aromatic rings	25%
3.	Direct N-glucuronidation at the carbamoyl side-chain	15%
4.	Substitution of the six-membered rings with sulphur-containing groups	5%

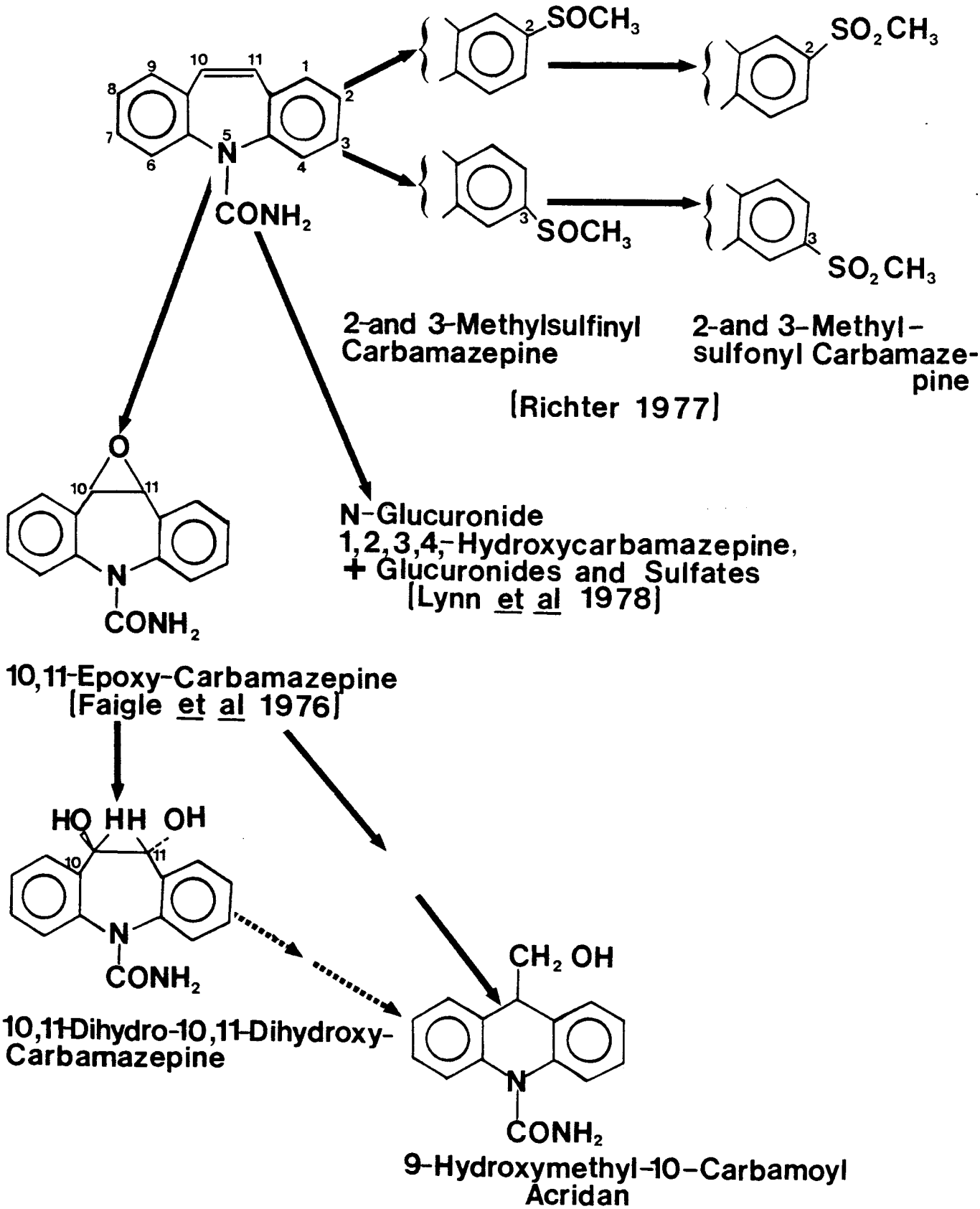


Fig. 5. Major pathways for the biotransformation of carbamazepine in man.

1.3.6.5. Elimination

Only some 2% of the dose of carbamazepine is excreted unchanged in the urine (Faigle et al 1976); this means therefore that carbamazepine is eliminated from the body chiefly by biotransformation.

The half-life, $T_{\frac{1}{2}}$, is the parameter that gives the best indication of the elimination rate. In the case of carbamazepine, half-lives ranging from 24 to 46 hours were found in volunteers (Palmer et al 1978; Rawlins et al 1975). However, Eichelbaum et al (1975), in their study on four epileptic patients, showed that the half-life of carbamazepine decreased from 35 to 20 hours after a few weeks' treatment. This was confirmed by Morselli et al (1975) who showed that the post-steady state plasma half-lives were approximately 15 hours in epileptic patients. The results from these studies suggest that carbamazepine induces its own metabolism (autoinduction).

Unlike phenytoin, carbamazepine is eliminated by dose-independent kinetics (Levy et al 1975; Rawlins et al 1975) and therefore saturation of the enzymes responsible for elimination is unlikely to occur with the dosages used therapeutically.

Clearance is a measure of the amount of the drug eliminated per unit time. In two studies (Eichelbaum et al 1975; Rawlins et al 1975), the mean plasma clearances were 30 and 25 ml/min respectively. This suggests that a 'first pass' metabolism is negligible.

1.3.7. Putative Mode of action of carbamazepine

1.3.7.1. Effects on seizure tests

Carbamazepine displayed the ability to depress maximal electro-shock seizures (50-100 volts, 50 c.p.s. A.C., for 0,63 secs applied through corneal electrodes) in both rats and mice (Koella et al 1976). In rats the oral ED_{50} for suppression of tonic convulsions of the hind limbs was 10 mg/kg and in mice the ED_{50} for this test was 12 mg/kg. In this study carbamazepine was administered 1 hour prior to electroshock and it was found to be maximally effective for at least four hours.

In 'epilepsy models' involving chemically-induced seizures, the potency of carbamazepine was less pronounced (Koella et al 1976). In mice challenged with picrotoxin in intraperitoneal doses of 12 mg/kg, carbamazepine in oral doses as high as 300 mg/kg was found to be ineffective. Carbamazepine inhibited convulsions produced by penetetazole (80 mg/kg i.p.); the ED_{50} was about 40 mg/kg. However, the percentage of mice protected did not increase with larger doses of carbamazepine. Against strychnine-induced convulsions, oral doses of 300 mg/kg of carbamazepine were not sufficient to inhibit convulsions completely.

Julien and Hollister (1975) investigated the effect of penicillin-induced epileptiform discharge and estrogen-induced spike-wave discharge (all in cats) and, in Rhesus monkeys, aluminium-oxide-induced behavioural and electrographic seizures.

It was found that while carbamazepine in doses of 2,5 mg/kg significantly reduced penicillin-induced discharges, doses of 5 mg/kg virtually abolished such discharges. Blood levels of carbamazepine were in the range of 4-6,5 µg/ml. In no animal was a blood level greater than 9 µg/ml required to suppress epileptiform discharge.

In the case of estrogen-induced epileptiform discharge, carbamazepine even in doses up to 30 mg/kg (blood levels greater than 15 µg/ml) was only partially effective in reducing the spike-wave discharge.

In three Rhesus monkeys with aluminium-oxide-induced behavioural and electrographic seizures, carbamazepine (20 mg/kg, i.m. daily, blood levels of 4-8 µg/ml) suppressed all convulsant activity in each of the three animals and returned the EEG patterns to normal. Such effect was reversible, and seizures returned within 10-16 days after cessation of drug administration.

1.3.7.2. Cerebrospinal effects

Following electrical stimulation of various structures belonging to the limbic system (e.g. the hippocampus), after-discharges can be recorded in the stimulated structure itself and in other areas interconnected with the challenged substrate.

Koella et al (1976) investigated the effect of carbamazepine, diazepam and diphenylhydantoin on the hippocampal after-discharge induced by electrical stimulation of the hippocampus. Carbamazepine (3 mg/kg i.v.) brought about almost complete blockade of this limbic epileptic seizure. This effect of

carbamazepine was found to be superior to that of diazepam and diphenyhydantoin. This could explain the therapeutic value of carbamazepine in temporal lobe seizures.

Holm et al (1970) identified a rather specific action of carbamazepine (20 mg/kg i.p., 6-9 µg/ml) on transmission of impulses through the nucleus ventralis anterior of the thalamus. This may be significant since the nucleus ventralis anterior has been implicated in the generation and spread of seizure discharge (Julien and Hollister 1975).

At the spinal level, Krupp (1969) and Theobald (1970) found that intravenous doses of carbamazepine (20 mg/kg) significantly reduced post-tetanic potentiation (PTP), but such effect was much less reduced at therapeutic doses (10 mg/kg). These findings were confirmed by Julien and Hollister (1975) who found that PTP could be blocked at doses above the therapeutic range in non-anaesthetized spinal cats.

Carbamazepine has also been shown to depress synaptic transmission in the spinal trigeminal nucleus. This action could explain its therapeutic effectiveness in trigeminal neuralgia (Fromm and Killian 1967).

1.3.7.3. Membrane effects

Schauf et al (1974) showed that carbamazepine at a concentration of 0,5 mM decreased the sodium and potassium conductances by 50% and 40%, respectively, in voltage-clamped Myxicola giant axons. In addition, membrane leakage conductance was reduced and the membrane was reversibly depolarised by 0-10 mV. There was little or no effect of carbamazepine on the time constants for sodium activation or inactivation or

potassium activation.

This behaviour is in contrast to that reported by Lipicky et al (1972) for diphenylhydantoin which was shown to have a relatively specific inhibitory effect on sodium conductance in squid axons.

The anticonvulsant activity of carbamazepine could therefore be explained by its ability to depress excitability rather than a highly specific effect on membrane component.

1.3.7.4. Biochemical effects

Gamma-aminobutyric acid

It has been shown that inhibition of the enzyme responsible for the synthesis of gamma-aminobutyric acid (GABA) or an action at postsynaptic sites to block GABA inhibitory action, leads to generalised seizures (Wood 1975; Meldrum 1975). Therefore it may be suggested that anticonvulsant drugs exert their therapeutic effects by raising the level of GABA in the central nervous system.

Post et al (1980) reported on a study carried out to assess the effects of carbamazepine on GABA metabolism. Nine patients with manic depressive illness were studied. GABA levels were measured in cerebrospinal fluid (CSF) before and during treatment with carbamazepine. The dose of carbamazepine ranged from 800-1 600 mg per day giving blood levels of 8-12 µg per ml. Compared to medication free values, GABA levels in CSF were not significantly altered by an average of 30 days' treatment with carbamazepine. Therefore it appears that carbamazepine does not have a major effect on

brain GABA as a mechanism of its anticonvulsant action.

Noradrenaline

Purdy et al (1977) investigated the effect of carbamazepine on the uptake and release of tritiated noradrenaline (^{3}H-NA). At concentrations in the therapeutic range (10^{-5}M), it was found that carbamazepine exhibited an 18% inhibition of ^{3}H-NA uptake in rabbit brain synaptosomes in the absence of effects on transmitter release. From a comparison of carbamazepine with imipramine, the authors concluded that the observed effects of carbamazepine were insufficient to account for the anticonvulsant action of the drug but the inhibition of the uptake at 10^{-5}M could perhaps explain carbamazepine's analgesic action.

Cyclic 3'5'-adenosine monophosphate (cAMP)

The cAMP content of cortical slices incubated in vitro were increased by the convulsant action of pentylenetetrazol (Lewin et al 1970), by electrical stimulation (Kakiuchi et al 1969), and by ouabain (Shimuzu et al 1970), and adenosine (Sattin and Rall 1970), both of which are epileptogenic when injected into the cortex. cAMP has also been shown to produce seizures when injected intraventricularly (Gessa et al 1970), and epileptiform discharge when applied to the surface of the cerebral cortex (Walker et al 1975).

Lewin and Bleck (1977) reported on a study carried out to investigate the effect of carbamazepine, phenytoin and phenobarbitone on the cAMP accumulation in rat cortical slices.

Carbamazepine (1 mM) and phenytoin (0,3 mM) inhibited the increase in cAMP produced by ouabain. Carbamazepine also antagonised the action of adenosine stimulation of cAMP accumulation whereas phenytoin did not. Both carbamazepine and phenobarbitone inhibited cAMP accumulation produced by noradrenaline. Phenytoin had no effect.

The authors concluded that if cAMP played a role in epileptogenesis, then inhibition of cAMP accumulation may well explain the mechanism of action of anticonvulsant drugs.

CHAPTER TWO

PATIENTS AND METHODS

CHAPTER TWO

2.1. Patient selection

A total of 23 epileptic patients took part in the study (12 males and 11 females). All patients had an established diagnosis of either generalised epilepsy (tonic-clonic seizures) or partial epilepsy (with complex symptomatology).

The patients were divided into two groups according to the frequency of their seizures:

RESPONDERS: Those patients who had no seizures in the month prior to entry into the study.

NON-RESPONDERS: Those patients who had at least one seizure a week in the month prior to entry into the study.

Compliance with medication was judged by the following criteria: (i) history and confirmation by random blood sampling and (ii) residence in an institution with documented ingestion by trained staff. It was also established that all patients had been receiving carbamazepine for not less than a month either alone or in combination with other anticonvulsants. This was done to ensure steady state levels of the drug in plasma. Furthermore, confirmation of therapeutic levels of carbamazepine in sera from non-responders was a condition of selection for the study.

Full clinical details appear in Tables 1 and 2 for responders and non-responders respectively.

Prior to commencement of the study the following base-line parameters were recorded in all patients:

(a) Electroencephalogram (EEG)

(b) Routine "12-channel analysis" for biochemical parameters

(c) Blood count

In addition, a Venereal Research Laboratory Test (VDRL) and computer-assisted tomography (CT-Scan) were carried out in certain patients not responding to therapy if the neurologists thought it warranted.

Details of all the above tests appear in the Appendix section.

2.2. Medication

All patients in this study received carbamazepine (LABETHICA) tablets of 200 mg.

The dose (mg/day) of carbamazepine and other concurrent medication appears in Tables 3 and 4 for responders and non-responders respectively.

2.3. Blood and Cerebrospinal Fluid Sampling (CSF)

In all patients studied, serum concentrations of carbamazepine, and its epoxy and dihydroxy metabolites were investigated by taking blood samples every two hours between 8 a.m. and 6 p.m. The first sample was taken just before the morning dose of carbamazepine. The dosage (mg/day) and the dosage interval were not altered in any of the patients.

CSF samples were taken only from the non-responders, at the discretion of the neurologist. Full consent was obtained from the patient before sampling. Serum and CSF samples were stored at -20°C until analysis.

2.4. Side effects

Assessment of the side effects of carbamazepine that occurred between 8 a.m. and 6 p.m. were made; full details appear in the Results section.

2.5. Length of period of observation

At the discretion of the neurologist, certain of the non-responders were hospitalised for a period of one to two weeks in order to find a drug regimen that would improve their seizure frequency before being discharged.

Responders were discharged after the last blood sample had been taken.

2.6. Location

The clinical investigations were carried out either at the Neurology Department at Groote Schuur Hospital (GSH), Cape Town, or at Valkenberg Mental Hospital, Cape Town.

The assay of carbamazepine and its metabolites was carried out at the Pharmacology Department, University of Cape Town Medical School.

2.7. Ethical control of the study

The study was only initiated after the approval of the Ethical Review Committee of the University of Cape Town Medical School.

2.8. Analytical Methodology

2.8.1. Introduction

The use of fluorimetry in combination with thin-layer chromatography, for the determination of carbamazepine and metabolites, was first reported by Scheiffart et al (1966). These authors applied biological fluids (CSF, plasma, urine, bile) or their dichloroethane extracts to silica plates, which were developed with carbon tetrachloride: methanol::7:1. Under ultraviolet light localised spots were scraped and eluted with 70% perchloric acid followed by heating at 120°C for 20 minutes. The fluorescence that developed in the eluate by this treatment was determined with a fluorescence spectrometer using an excitation wavelength of 358 nm and an emission wavelength of 498 nm. The concentration of carbamazepine was calculated from a calibration curve.

A similar but rather less tedious procedure was described by Christiansen (1973). 10 µl of diluted plasma was applied directly to silica plates. The chromatograms were developed (solvents: benzene: dioxane: ethanol: 25% NH_4OH::5:4:1:1), dried, dipped into perchloric acid reagent (70% $HClO_4$ 12 ml, ethanol 150 ml, H_2O 130 ml), and then heated at 108°C for 8 minutes. The fluorescence was measured in situ by direct scanning of spots at 498 nm with a fluorescence densitometer (Vitatron TLD 100 flying spot scanner) using an excitation wavelength of 366 nm. A calibration curve was made with the standards.

Meilink (1974) applied a dichloroethane extract onto thin layer plates which were then developed in ethyl acetate. The spots were scraped, eluted and induction of fluorescence of carbamazepine and its metabolites carried out by use of ammonium-ceric sulphate and phosphoric acid reagent. Measurement was made with a fluorescence spectrometer; the excitation wavelength was 400 to 408 nm and the emission wavelength was 475 nm to 480 nm. Cyproheptadine, imipramine, protrytaline and quinine, among others, were found to interfere with this method.

The method used in this study is based on that of Hundt and Clark (1975). In this method carbamazepine and metabolites were determined simultaneously, only 2 µl of serum or CSF being required for a duplicate determination and no extraction procedure being involved. The separated spots (developing solvent was ethyl acetate: benzene: methanol::5:4:1) were converted into fluorescent compounds by exposing the plates to hydrogen chloride gas for 5 minutes and then to ultraviolet radiation from a mercury lamp. The fluorescence was measured quantitatively using a spectrofluorimeter (Perkin-Elmer MPF3) equipped with a thin-layer chromatograph scanning attachment. Excitation wavelength was 354 nm, emission wavelength 486 nm and emission filter 430 nm. A calibration curve was constructed from standards.

2.8.2. Experimental

2.8.2.1. Reagents

Carbamazepine, 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine were obtained from Ciba-Geigy, Johannesburg, South Africa. Benzene, ethyl acetate, methanol, concentrated sulphuric acid and concentrated hydrochloric acid were guaranteed reagent grade (Analar, B.D.H. Chemicals, Poole, England).

2.8.2.2. Apparatus

A Zeiss KM3 Chromatogram Spectrophotometer was used to measure the fluorescence of the spots on the thin layer plates. The following operating conditions were used:

(i) INDICATOR UNIT:

Mode selector set to "R"

Recording mode selector to KOMP F

Damping switch set to "1"

High-voltage selector to step 1

(ii) RECORDER UNIT:

Chart paper speed 5 cm/min

Scanning speed 100 mm/min

Measuring range 1V

(iii) ILLUMINATOR AND MONOCHROMATOR:

Mercury source was provided

Exciting wavelength set to 257 nm

Slit width set to 0,5 mm

ILLUMINATOR AND MONOCHROMATOR:

Barrier filter FL46 was used

Slip-in diaphragm 3,5 mm was used

The amplification was adjusted to obtain approximately 90% full-scale deflection on the recorder when the strongest spot in the chromatogram was being scanned.

The other apparatus used consisted of silica gel 60 TLC plates, dimensions 10 x 20 cm (Merck), a sample applicator for thin layer chromatography (EVA CHROM), a 2 µl capillary (Minicaps), a universal UV lamp, type 29000 (Camag), and a Sartorius electronic microbalance. (See Appendix F for suppliers.)

2.8.2.3. Stock solutions

A single stock solution containing 1 mg of carbamazepine, 0,33 mg of 10,11-epoxy-carbamazepine and 0,33 mg of 10,11-dihydro-10,11-dihydroxy-carbamazepine in 10 ml absolute methanol was prepared as follows. The reagents were weighed separately on a Sartorius electronic microbalance and solutions made up with methanol to contain 3 mg of carbamazepine, 1 mg of 10,11-epoxy-carbamazepine and 1 mg of 10,11-dihydro-10,11-dihydroxy-carbamazepine per 10 ml of solution. Equal volumes of these solutions were then added together so as to give the final stock solution.

Final stock solution used for CSF standards contained 0,587 mg of carbamazepine, 0,336 mg of 10,11-epoxy-carbamazepine and 0,253 mg of 10,11-dihydro-10,11-dihydroxy-carbamazepine in 10 ml of absolute methanol.

2.8.2.4. Standard Solutions

Four standard solutions were prepared by adding with micropipettes 20, 40, 80 and 120 µl of the stock solution in each instance to sample tubes. The solvent was evaporated off, and the residue then re-dissolved in 1 ml serum/CSF so as to obtain the standard serum/CSF solutions.

2.8.2.5. Spotting the Plates

To 200 µl of serum (patient or standard) was added 200 µl of distilled water. After thoroughly mixing on a vortex mixer exactly 2 µl of the serum solution was then spotted on the plate in a single, smooth application. In this fashion, twenty-two spots with unknown serum and standard serum alternating in duplicate were applied to a single 10 x 20 cm plate. This corresponded to six unknown serum samples being processed in duplicate. 2 µl of CSF (patient or standard) was applied directly to the plate without dilution.

2.8.2.6. Chromatography

The eluent used was ethylacetate : benzene : methanol:: 5:4:1. The development was carried out in an unsaturated tank up to a height of 8 cm; the elution time was ca. 20 minutes.

After drying them briefly with a hairdryer, the plates were dried at 100°C for 5 minutes and then exposed for 15 minutes to hydrogen chloride gas generated in a chromatographic tank by pipetting 3 ml of concentrated hydrochloric acid into 20 ml of concentrated sulphuric acid in a small beaker in the tank. Immediately after exposure to hydrogen chloride gas, the plates were irradiated for 1 hour with ultra-violet light

obtained from an unfiltered mercury lamp (254 nm). This procedure converted carbamazepine and its metabolites into fluorescent compounds that could be measured directly and quantitatively with the Zeiss KM3 chromatogram spectrophotometer. Each series of spots corresponding to an applied sample was scanned in the direction of solvent flow.

From the peak heights obtained for the known concentrations of carbamazepine and metabolites, an equation of the best straight-line fit was obtained by linear regression analysis, and this equation was used in the calculation of the concentrations of carbamazepine and metabolites in unknown sera and CSFs.

Figs. 11 and 12 represent part of a chromatogram showing the peaks obtained for standard serum and CSF containing the indicated amounts of carbamazepine and its two metabolites.

2.8.2.7. Validation

Prior to the patient samples being assayed, four serum and CSF samples were spiked at each concentration in order to ensure reproducibility. From the regression coefficients obtained (Tables 5 - 10), it can be seen that this ratio is linear over the concentration range used. The coefficient of variation for carbamazepine standards, in serum and CSF, did not exceed 3% at each concentration and for the metabolites the coefficient of variation was within 8% at each concentration employed.

If the peak heights of duplicate samples did not agree to within 10% the samples were re-assayed.

Furthermore, a control serum (EMIT-AED, SYVA) was run with the assay and if the carbamazepine concentration did not agree to within 10% of the actual value, the standards were recalibrated.

Control serum for the metabolites and control CSF for carbamazepine and metabolites were also prepared and if agreement in concentration between the calculated and actual value did not agree to within 10%, the standards were recalibrated. Calibration curves for carbamazepine and metabolites in serum are shown in figs. 6-8; for CSF in figs. 9-11.

2.8.2.8. Specificity

Hundt and Clark (1975) in their paper reported that sera of a large number of patients receiving a diversity of drugs were chromatographed and treated as already described. In no instance was any spot found that could possibly interfere in the assay of carbamazepine or the two metabolites.

In this study blank serum samples were run with each assay. No interfering peaks could be detected.

The Pharmacology Department, Medical School, University of Cape Town, employs this method for the routine assay of carbamazepine. Serum samples from patients, not on carbamazepine therapy, have been shown to give no peaks when the chromatographic plates are scanned either on the Perkin-Elmer MPF3 spectrofluorimeter or the Zeiss KM3 spectrophotometer.

2.8.2.9. Sensitivity

According to Hundt and Clark (1975), the lowest demonstrable concentration of carbamazepine in serum was 0,1 µg/ml, while those for each metabolite corresponded to 0,05 µg/ml.

In this study no patients had carbamazepine concentrations less than 2 µg/ml and metabolite concentrations less than 0,1 µg/ml.

2.9. Calculation of Area under the Curve (AUC)

The area under the plasma concentration/time curve for carbamazepine (AUC_{CBZ}), 10,11-epoxy-carbamazepine (AUC_{EPOXY}) and 10,11-dihydro-10,11-diydroxy-carbamazepine (AUC_{DI-OH}) were calculated by making use of the trapezoidal rule (see Appendix E).

2.10. Statistical analysis

The Mann-Whitney U test was used for the comparison of data between responders and non-responders. The Mann-Whitney is one of the most powerful non-parametric tests and was employed because the distribution of parameters from the assay and patient population was not expected to be normal. The power efficiency of the test is close to 95 per cent (Mood 1954).

Other statistical testsused included the Spearman rank correlation test, the randomization test for two independent samples and the Student's t distribution. (Details of all the above tests appear in the Appendix.)

TABLE 1: CLINICAL DATA OF EPILEPTIC RESPONDERS.

INITIALS	SEX	AGE (YRS)	WEIGHT (KG)	RACE	DURATION OF EPILEPSY (YRS)	TYPE OF EPILEPSY	TYPE OF SEIZURE	FREQUENCY OF SEIZURE (PER MONTH)
AvA	F	20	70	W	9	GE	GM	NIL
VP	F	35	50	C	10	GE	GM	"
RI	F	18	52	C	6	GE	GM	"
KS	M	27	76	C	11	PE	PM	"
JA	M	38	75	C	14	PE	PM	"
JT	M	21	62	C	5	PE	PM	"
RT	M	33	83	W	27	GE	GM	"
DN	M	27	65	W	14	GE	GM	"
DH	M	42	72	W	11	GE	GM	"
SS	M	34	78	W	10	PE	PM	"
BR	F	32	60	W	28	PE	PM	"
MA	M	23	62	W	3	PE	PM	"

GE: Generalised epilepsy
PE: Partial epilepsy
GM: Grand-mal seizures
PM: Psychomotor seizures
M: Male
F: Female
W: White
C: Coloured

TABLE 2: CLINICAL DATA OF EPILEPTIC NON-RESPONDERS.

INITIALS	SEX	AGE (YRS)	WEIGHT (KG)	RACE	DURATION OF EPILEPSY (YRS)	TYPE OF EPILEPSY	TYPE OF SEIZURE	FREQUENCY OF SEIZURE (PER MONTH)
MF	F	32	51	W	14	PE	PM	8
BG	F	33	53	W	24	PE	PM	8
GP	F	20	52	C	11	GE	GM	10
RA	M	17	74	W	5	PE	PM	7
MC	M	45	68	W	20	PE	PM	12
QJ	F	18	43	C	13	GE	GM	13
MB	M	19	54	C	4	GE	GM	6
JN	F	20	49	W	18	GE	GM	10
CC	F	16	41	C	9	GE	GM	6
RC	M	44	75	C	12	GE	GM	6
SH	F	10	40	W	5	PE	PM	6

GE: Generalised epilepsy
PE: Partial epilepsy
GM: Grand-mal seizures
PM: Psychomotor seizures
M: Male
F: Female
W: White
C: Coloured

TABLE 3: MEDICATION CHART OF EPILEPTIC RESPONDERS.

INITIALS	CARBAMAZEPINE (mg/day)	PHENOBARBITONE (mg/day)	PHENYTOIN (mg/day)	CO-MEDICATION (mg/day)
AvA	1 000	-	-	-
VP	800	-	-	-
RI	400	-	-	-
KS	600	-	300	CLOZAPINE (200)
JA	400	-	200	CLOZAPINE (200)
JT	600	-	-	-
RT	600	-	-	CHLORPROMAZINE (150) CLOZAPINE (200)
DN	400	-	-	FLUPHENAZINE (25)
DH	400	-	-	FLUPHENAZINE (25)
SS	400	-	-	CHLORPROMAZINE (100) CLOZAPINE (200)
BR	1 200	-	-	-
MA	400	-	-	CHLORPROMAZINE (700) ORPHENADRINE (200)

TABLE 4: MEDICATION CHART OF EPILEPTIC NON-RESPONDERS.

INITIALS	CARBAMAZEPINE (mg/day)	PHENOBARBITONE (mg/day)	PHENYTOIN (mg/day)	CO-MEDICATION (mg/day)
MF	800	-	-	DOMPERIDONE (30) MAGNESIUM TRISILICATE (1 000)
BG	800	-	-	-
GP	800	60	-	-
RA	800	-	-	ETHOSUXIMIDE (800)
MC	900	-	-	-
QJ	800	60	200	-
MB	800	-	100	-
JN	1 000	90	-	-
CC	600	90	-	-
RC	800	-	300	-
SH	600	-	-	-

TABLE 5: VALIDATION OF ANALYTICAL METHOD FOR CARBAMAZEPINE IN SERUM.

EXPECTED CONCENTRATION (µg/ml)	NUMBER OF SPIKED SAMPLES	MEAN PEAK HEIGHT (mm)	STANDARD DEVIATION	COEFFICIENT VARIATION (%)	OBSERVED CONCENTRATION (µg/ml)
0	4	0	0	0	0,00
2	4	32,5	0,58	1,78	1,99
4	4	65	1,15	1,78	4,04
8	4	130,3	2,75	2,11	8,15
12	4	189,5	1,91	1,00	11,9

Slope = 15,9
Y Intercept = 1,01
Correlation coefficient (r) = 0,9996
r^2 = 0,9992

TABLE 6: VALIDATION OF ANALYTICAL METHOD FOR 10,11-EPOXY-CARBAMAZEPINE IN SERUM.

EXPECTED CONCENTRATION (µg/ml)	NUMBER OF SPIKED SAMPLES	MEAN PEAK HEIGHT (mm)	STANDARD DEVIATION	COEFFICIENT VARIATION (%)	OBSERVED CONCENTRATION (µg/ml)
0	4	0	0	0	0
0,67	4	36,3	0,96	2,64	0,66
1,33	4	74,3	0,50	0,67	1,36
2,67	4	146,8	2,63	1,79	2,69
4,00	4	217,5	4,72	2,17	3,99

Slope = 54,4
Y Intercept = 0,47
Correlation coefficient (r) = 0,9996
r^2 = 0,9992

TABLE 7: VALIDATION OF ANALYTICAL METHOD FOR 10,11-DIHYDRO-10,11-DIHYDROXY-CARBAMAZEPINE IN SERUM.

EXPECTED CONCENTRATION (µg/ml)	NUMBER OF SPIKED SAMPLES	MEAN PEAK HEIGHT (mm)	STANDARD DEVIATION	COEFFICIENT VARIATION (%)	OBSERVED CONCENTRATION (µg/ml)
0	4	0	0	0	0
0,67	4	11	0,71	6,45	0,64
1,33	4	23,5	0,58	2,47	1,36
2,67	4	46,3	1,50	3,23	2,69
4,00	4	68,5	5,25	7,67	3,98

Slope = 17,2
Y Intercept = 0,04
Correlation coefficient (r) = 0,9961
r^2 = 0,9922

TABLE 8: VALIDATION OF ANALYTICAL METHOD FOR CARBAMAZEPINE IN CSF.

EXPECTED CONCENTRATION (µg/ml)	NUMBER OF SPIKED SAMPLES	MEAN PEAK HEIGHT (mm)	STANDARD DEVIATION	COEFFICIENT VARIATION (%)	OBSERVED CONCENTRATION (µg/ml)
0	4	0	0	0	0
1,17	4	27,9	0,85	3,04	1,20
2,35	4	54,4	1,25	2,29	2,35
4,69	4	106,8	0,96	0,90	4,63
7,04	4	163	4,32	2,65	7,07

Slope = 23
Y Intercept = 0,21
Correlation coefficient (r) = 0,9994
r^2 = 0,9988

TABLE 9: VALIDATION OF ANALYTICAL METHOD FOR 10,11-EPOXY-CARBAMAZEPINE IN CSF.

EXPECTED CONCENTRATION (µg/ml)	NUMBER OF SPIKED SAMPLES	MEAN PEAK HEIGHT (mm)	STANDARD DEVIATION	COEFFICIENT VARIATION (%)	OBSERVED CONCENTRATION (µg/ml)
0	4	0	0	0	0
0,68	4	21	1,63	7,76	0,68
1,35	4	41,8	0,96	2,29	1,39
2,69	4	80,8	0,96	1,19	2,70
4,04	4	120	0,96	0,80	4,01

Slope = 29,6
Y Intercept = 0,83
Correlation coefficient (r) = 0,9996
r^2 = 0,9992

TABLE 10: VALIDATION OF ANALYTICAL METHOD FOR 10,11-DIHYDRO-10,11-DIHYDROXY-CARBAMAZEPINE IN CSF.

EXPECTED CONCENTRATION (µg/ml)	NUMBER OF SPIKED SAMPLES	MEAN PEAK HEIGHT (mm)	STANDARD DEVIATION	COEFFICIENT VARIATION (%)	OBSERVED CONCENTRATION (µg/ml)
0	4	0	0	0	0
0,51	4	10,1	0,85	0,43	0,52
1,01	4	20,4	1,25	0,63	1,10
2,03	4	36,3	1,50	0,75	2,01
3,04	4	54,0	2,94	1,47	3,02

Slope = 17,5
Y Intercept = 1,06
Correlation coefficient (r) = 0,9962
r^2 = 0,9924

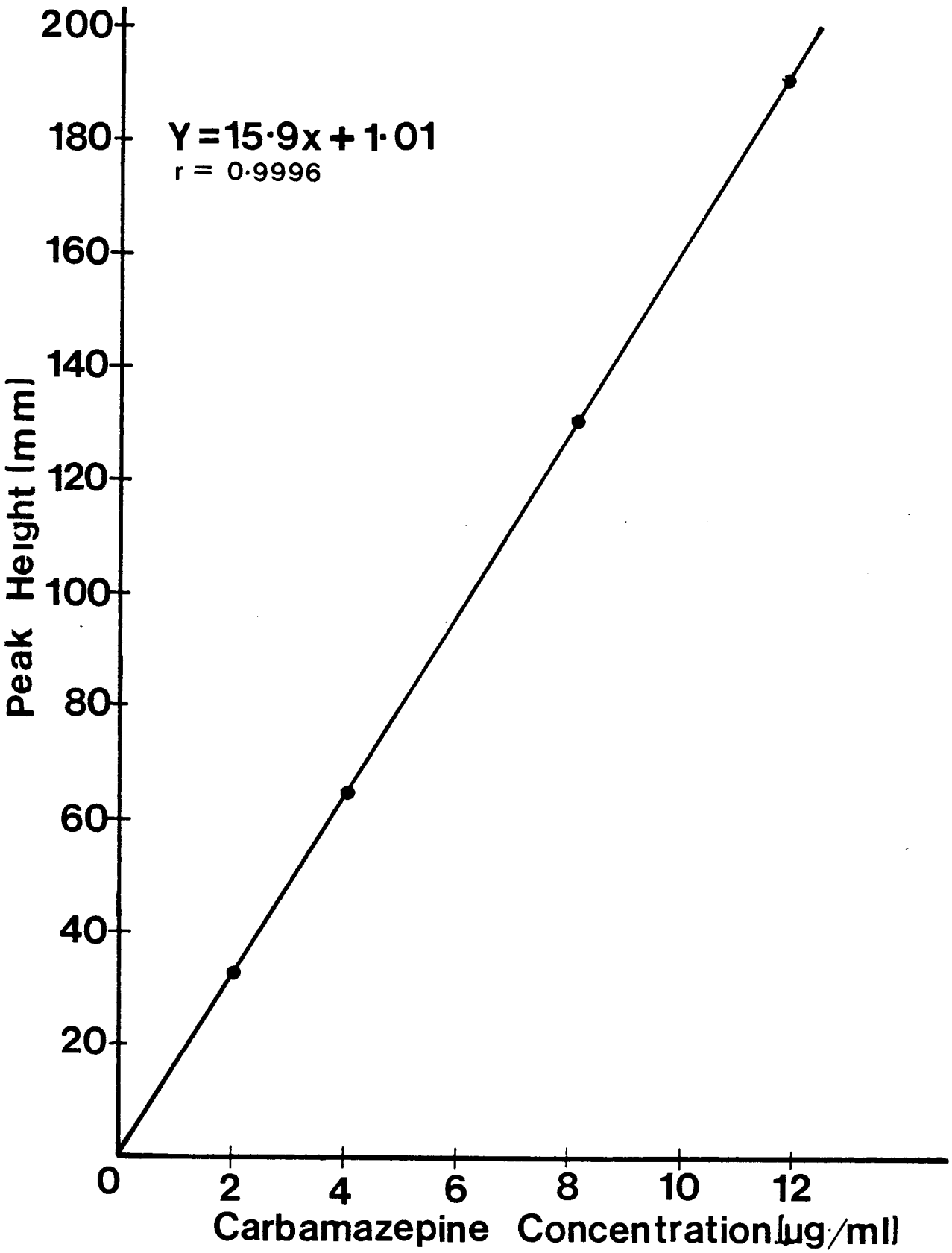


Fig. 6: Calibration curve for the determination of carbamazepine in serum. Co-ordinates plotted represent mean of observed valves.

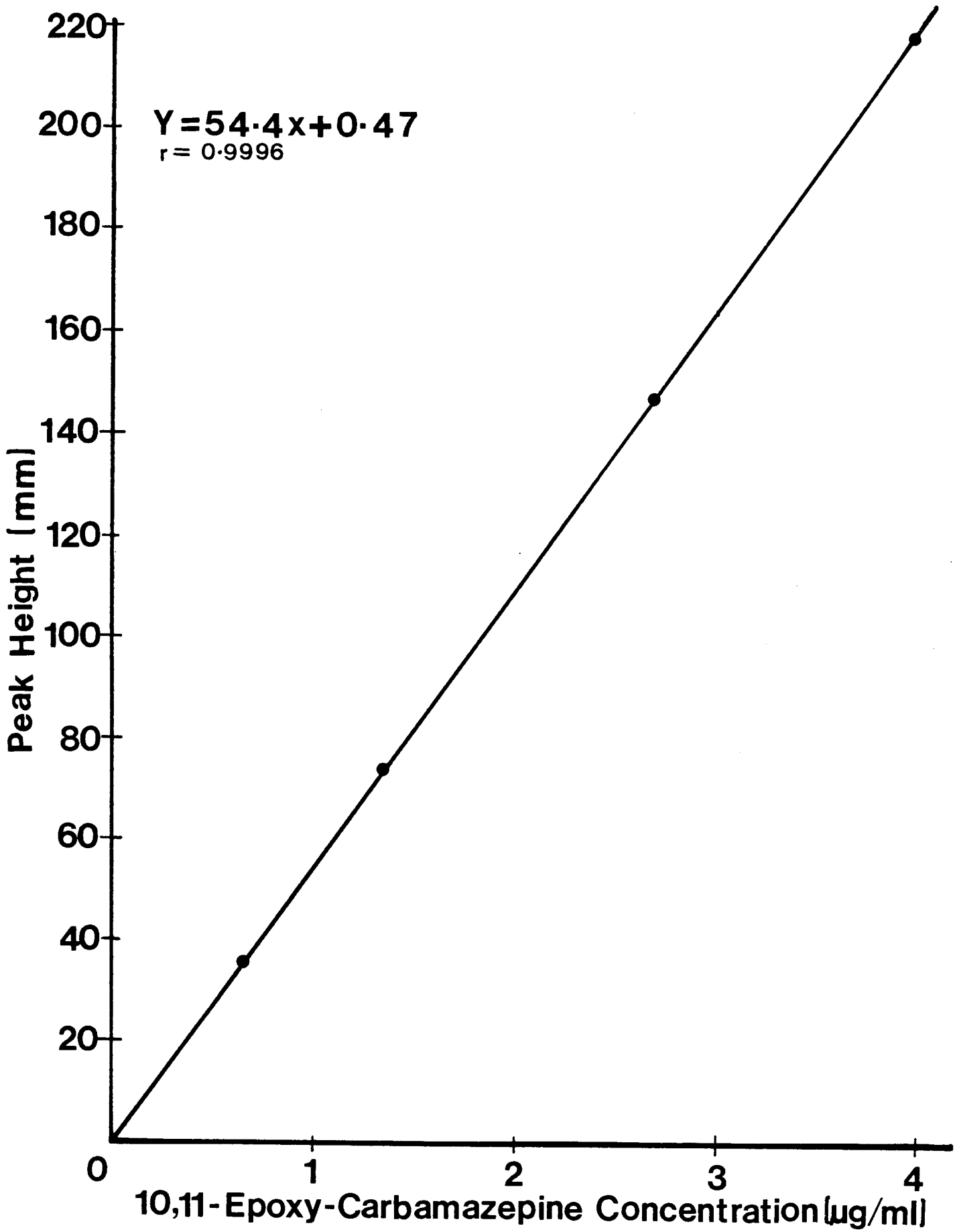


Fig. 7: Calibration curve for the determination of 10,11-Epoxy-Carbamazepine in Serum. Co-ordinates plotted represent mean of observed values.

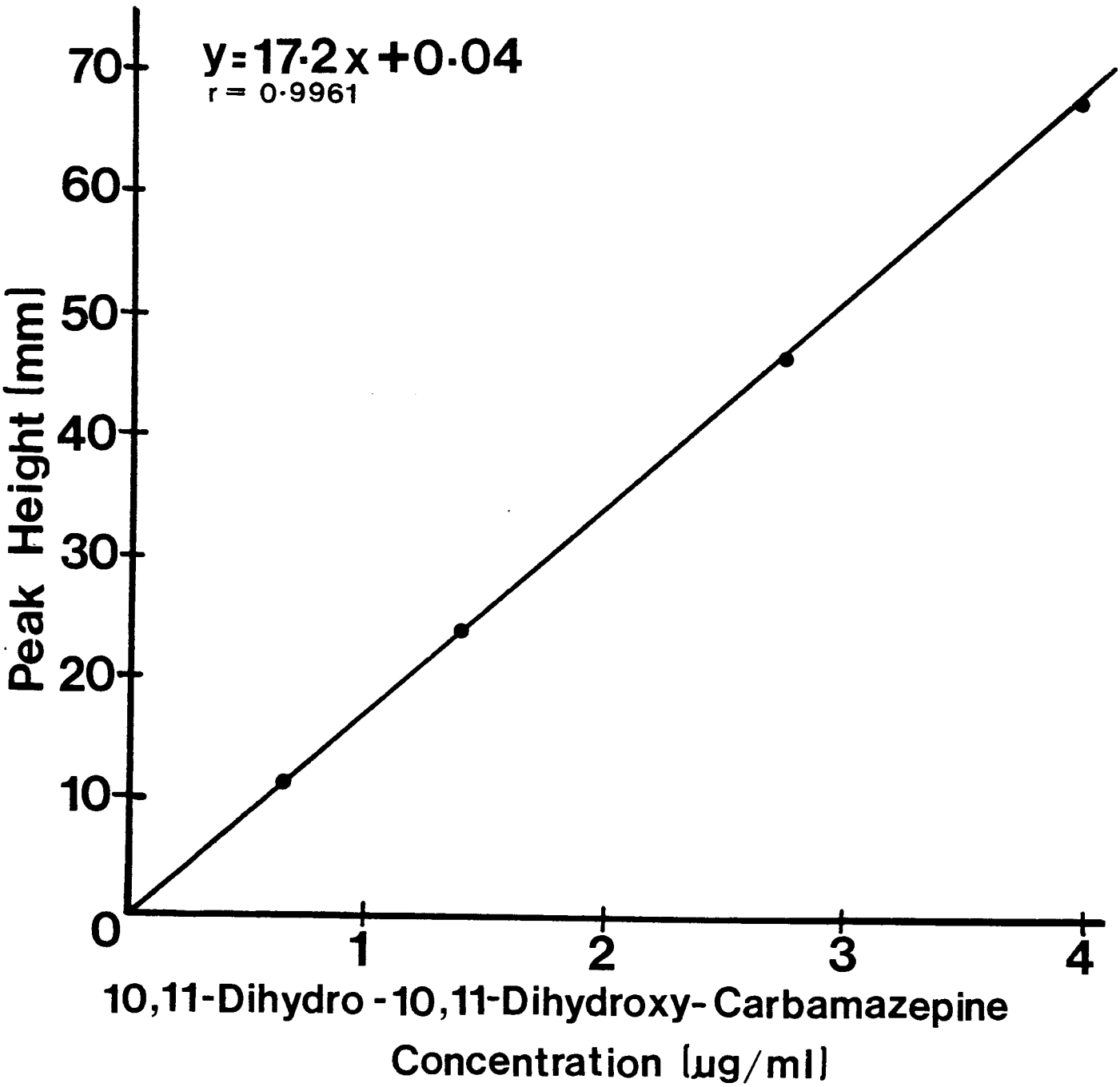


Fig. 8: Calibration curve for the determination of 10,11-Dihydro-10,11-Dihydroxy-Carbamazepine in serum. Co-ordinates plotted represent mean of observed values.

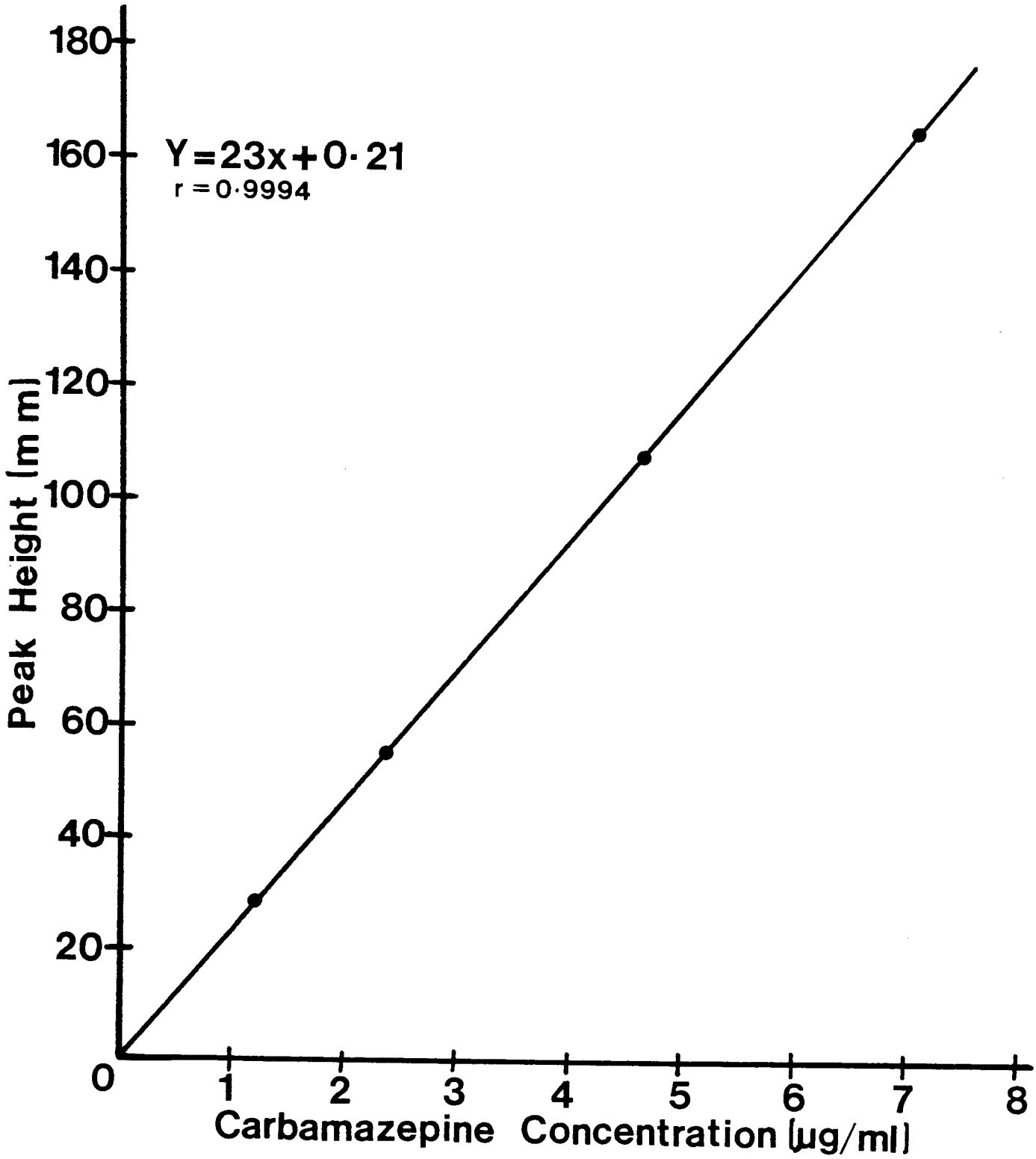


Fig. 9: Calibration curve for the determination of carbamazepine in CSF. Co-ordinates plotted represent mean of observed values.

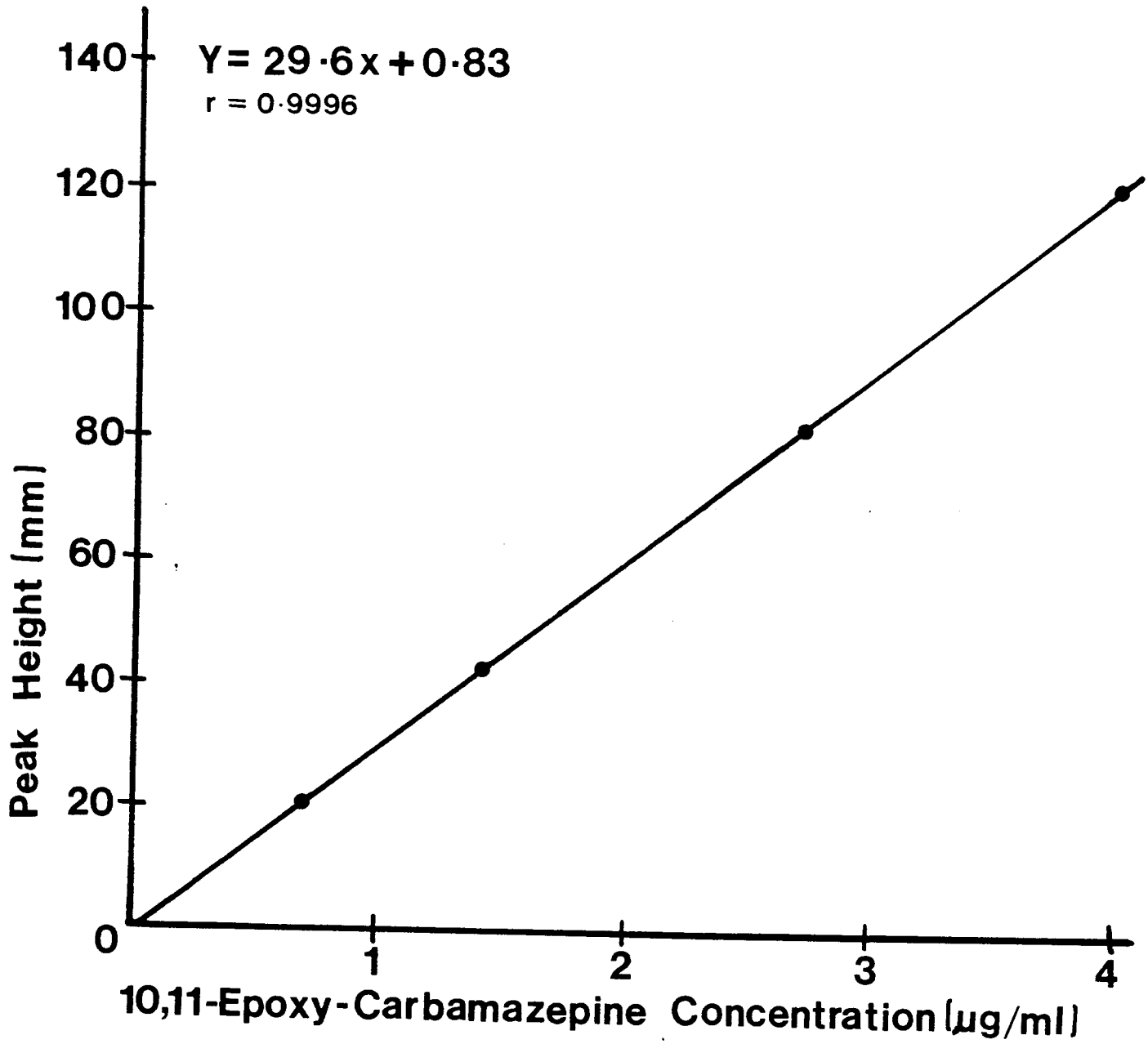


Fig. 10. Calibration curve for the determination of 10,11-epoxy-carbamazepine in CSF. Co-ordinates plotted represent mean of observed valves.

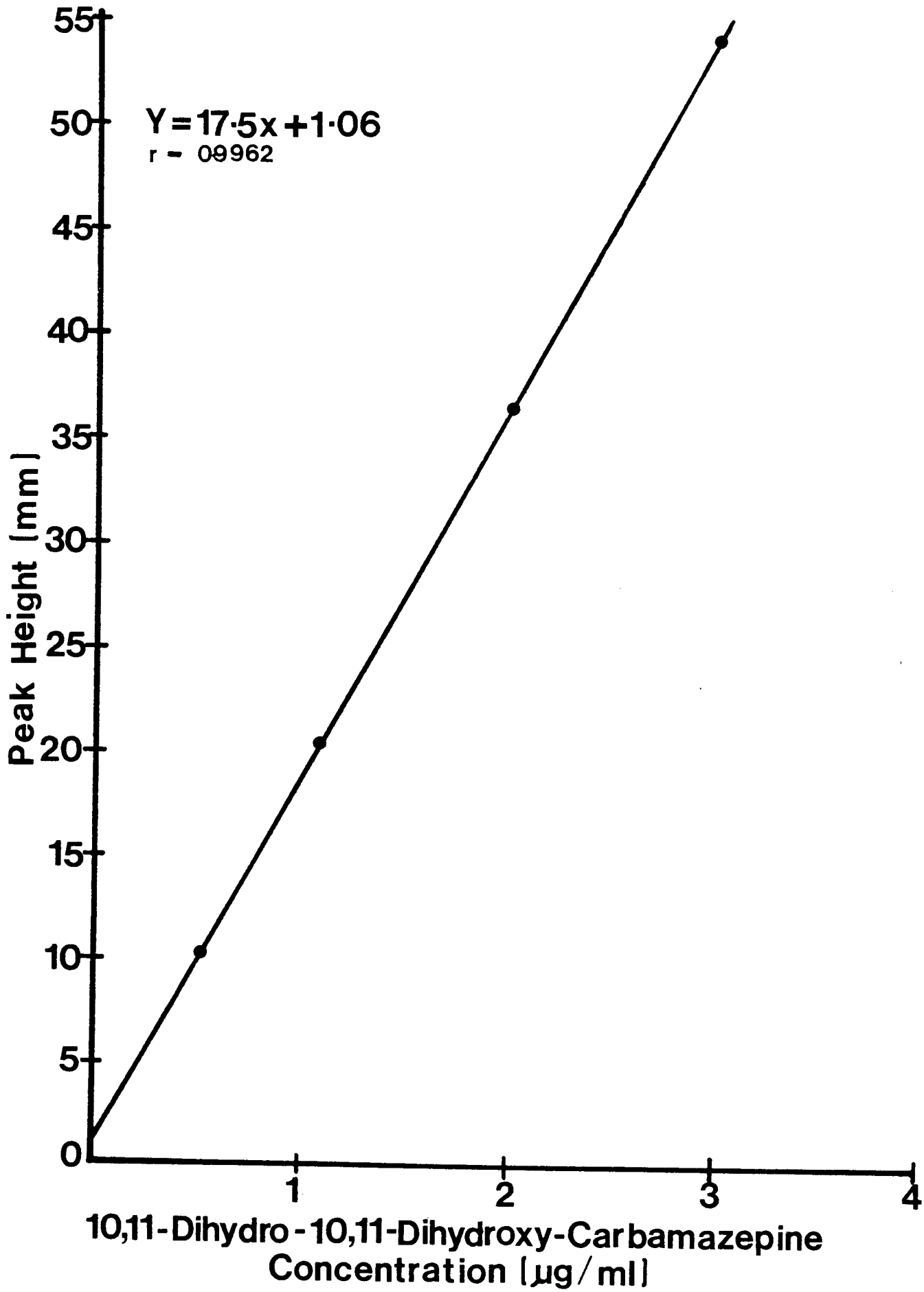


Fig. 11: Calibration curve for the determination of 10,11-Dihydro-10,11-Dihydroxy-Carbamazepine in CSF. Co-ordinates plotted represent mean of observed values.

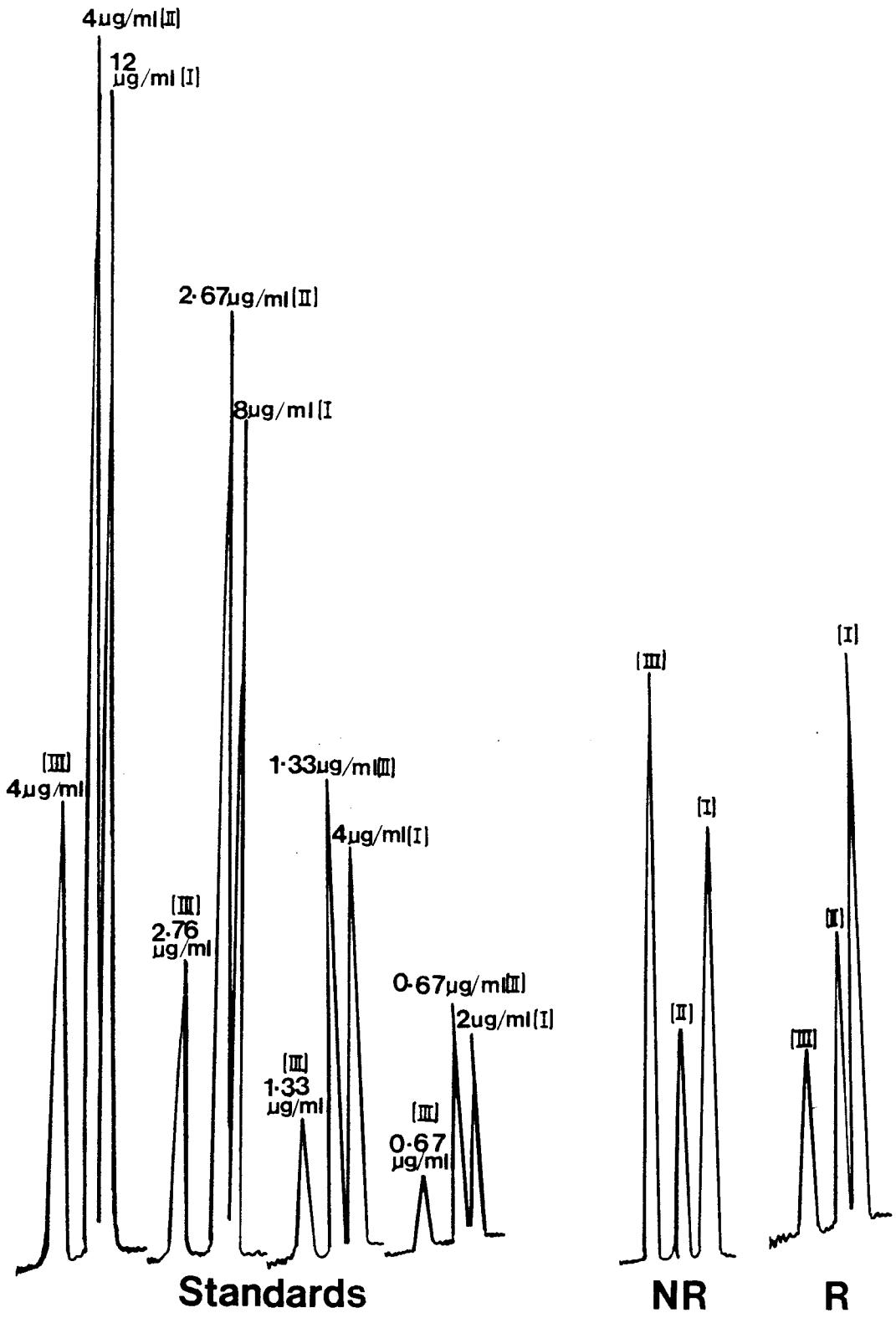


Fig. 12: Chromatogram of different amounts of carbamazepine and its two metabolites in serum. (I) carbamazepine; (II) 10,11-Epoxy-carbamazepine (III) 10,11-Dihydro-10,11-Dihydroxy-Carbamazepine; (NR) typical non-responder; (R) typical responder.

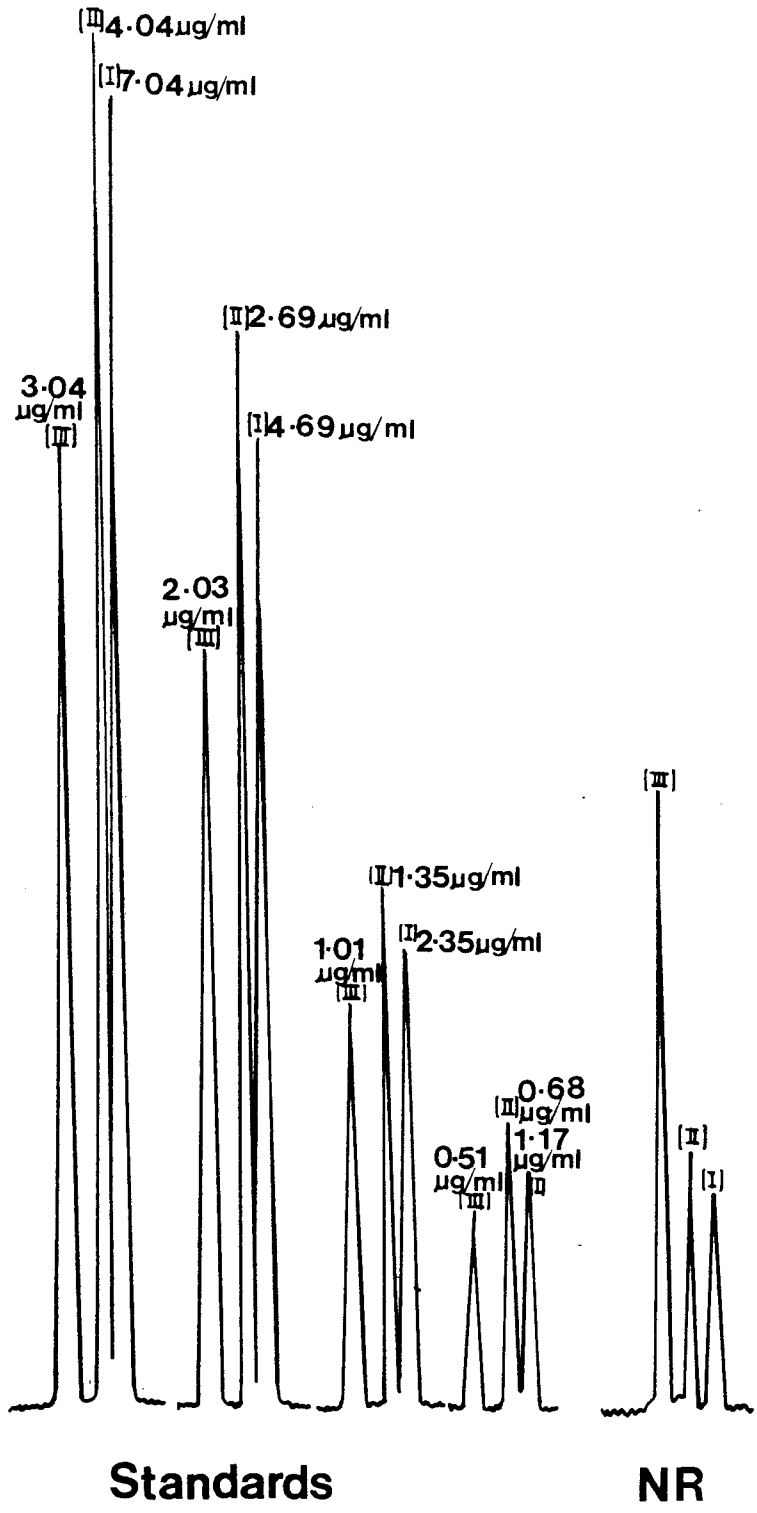


Fig. 13 Chromatogram of different amounts of carbamazepine and its two metabolites in CSF. (I) Carbamazepine; (II) 10,11-Epoxy-Carbamazepine (III) 10,11-Dihydro-10,11-Dihydroxy-Carbamazepine; (NR) Non-responder.

CHAPTER THREE

RESULTS

CHAPTER THREE

3.1. Results

3.1.1. Side-effects

Only 6 of the 23 patients in the study complained of side-effects; 3 patients in each of the responder and non-responder groups.

Headaches, nausea and drowsiness were the most frequently mentioned complaints. Table 11 gives details of carbamazepine and metabolite concentrations at which these side-effects occurred. In the responder group, the mean levels of carbamazepine and its epoxy and dihydroxy metabolites were 8,18, 1,33 and 2,53 µg/ml in patients with side-effects, compared with 6,11, 0,82 and 2,17 µg/ml in patients without side-effects. In the non-responder group, the mean levels of carbamazepine and its epoxy and dihydroxy metabolites were 8,99, 2,04 and 5,31 µg/ml in patients with side-effects, compared with 8,41, 1,73 and 5,19 µg/ml in patients without side-effects.

The randomization test for two independent samples showed that these results are not statistically significant.

3.1.2. Area under the curve (AUC) of carbamazepine and metabolites

In this study all patients had been taking carbamazepine for not less than a month before serum samples were collected. It can therefore be assumed that the steady-state concentrations had been reached. The AUC was therefore chosen as the parameter that would best give the overall picture of the concentrations of carbamazepine and its metabolites in plasma. Tables 12 and 13 give details of the AUC of carbamazepine and its epoxy and dihydroxy metabolites for responders and non-responders, respectively. This was calculated from the concentration/time profiles as illustrated by figs. 14 and 15 for responders and figs. 16 and 17 for non-responders. Tables 19 and 20 give details of the concentrations of carbamazepine and its metabolites between 8 a.m. and 6 p.m. in responders and non-responders, respectively.

In performing the Mann-Whitney U test on these results, it was found that there was no significant difference in the AUC_{CBZ} between responders and non-responders. However, the AUC_{DI-OH} was significantly higher in the non-responders ($P < 0,002$). The AUC_{EPOXY} was also higher in non-responders but at a lower significance level ($P < 0,02$).

The same results are obtained by using mean concentrations of carbamazepine and metabolites (Tables 14 and 15).

3.1.3. Polypharmacy versus carbamazepine monotherapy

Eight patients in this study were taking carbamazepine concomitantly with either phenobarbitone and/or phenytoin; 6 patients were from the non-responder group and 2 from the responder group. As these drugs are known to induce liver enzymes, a comparison of the AUC of the dihydroxy and epoxy metabolites was made between these patients and the 8 that were on carbamazepine monotherapy. No significant difference in metabolite concentration could be demonstrated between the two groups, presumably because auto-induction of metabolism had already proceeded to a limit.

Phenothiazines are a class of drugs that have the ability to inhibit the cytochrome P450 enzymes of the liver. This effect has been clearly demonstrated for thioridazine on the metabolism of phenytoin (Vincent 1980).

In the responder group, 7 out of the 12 patients were taking phenothiazines concomitantly with carbamazepine. However, no significant difference in the concentrations of the dihydroxy and epoxy metabolites could be demonstrated between these patients and those on carbamazepine monotherapy in that group.

3.1.4. Cerebrospinal fluid (CSF) concentrations of carbamazepine and metabolites

It was possible to obtain CSF samples from 7 out of the 11 patients classified as non-responders. Table 16 gives details of CSF and serum concentrations (obtained within the same time interval) for carbamazepine and metabolites. The ratio of CSF/serum gives a mean (±SD) free fraction for carbamazepine of 0,25±0,05; 0,62±0,13 for the 10,11-epoxy-carbamazepine and 0,41±0,08 for the 10,11-dihydro-10,11-dihydroxy-carbamazepine. These values are in agreement with those obtained by Schneider and Berenguer (1975). It is interesting to note that the mean (±SD) ratio of 10,11-epoxy-carbamazepine to carbamazepine in CSF is 0,55 (±0,13) whilst the mean (±SD) ratio of 10,11-dihydro-10,11-dihydroxy-carbamazepine is as high as 1,17 (±0,36). For ethical reasons, it was not possible to obtain CSF from the patients in the responder group. As a result, to what extent these findings are significant is not known.

3.1.5. Fluctuations in serum levels of carbamazepine

Two to four hours after each administration, a more or less pronounced peak in the concentration of carbamazepine in blood was attained in every patient. Tables 17 and 18 give a detailed review of the difference between minimum and maximum carbamazepine concentrations occurring in the course of the day between 8 a.m. and 6 p.m. in responders and non-responders, respectively.

In the responder group, the mean (±SD) minimum

concentration was 4,82±1,68 µg/ml, whereas the mean (±SD) maximum concentration was 6,86±1,86 µg/ml. In the non-responder group, on the other hand, the mean (±SD) minimum concentration of carbamazepine was 5,71±2,06 µg/ml and mean (±SD) maximum concentration was 8,78±2,81 µg/ml. Average fluctuations from the mean (±SD) were 36±20% and 45±13% in responders and non-responders, respectively. Figs. 14 and 15 and figs. 16 and 17 are graphical representations of these fluctuations.

3.1.6. Dose of carbamazepine

Although no significant difference could be demonstrated in the AUC_{CBZ} between responders and non-responders, the patients in the non-responder group had a higher mean (±SD) oral intake of carbamazepine 791(±114)mg/day compared with a mean (±SD) of 600(±270)mg/day for patients in the responder group ($P < 0,05$). The significance of this will be discussed later.

3.1.7. Correlation between the plasma level of carbamazepine and its metabolites

The relationship between the mean concentration of carbamazepine and its epoxy and dihydroxy metabolites are shown in figs. 18 to 21.

The correlation coefficient (r) of 0,76 ($P < 0,01$) and 0,88 ($P < 0,001$) in responders and non-responders, respectively, indicates that a strong positive correlation exists between the concentration of 10,11-epoxy-carbamazepine and the

concentration of carbamazepine in plasma of the patients in this study. However, in interpreting these correlations, it must be noted that the intercepts of the regression lines do not pass through zero. This means that there may not necessarily be a linear relationship between the concentration of the epoxy metabolite and the drug in plasma.

In the case of the 10,11-dihydro-10,11-dihydroxy-carbamazepine, again a strong positive correlation exists between the concentration of this metabolite and the concentration of carbamazepine; correlation coefficient (r) is 0,81 ($P<0,01$) and 0,85 ($P<0,001$) in responders and non-responders, respectively. The intercepts of the regression lines pass through zero and therefore a linear relationship between this metabolite and the drug may exist. However, since there may not be linear relationship between the epoxy metabolite and carbamazepine, it is unlikely that a change in the plasma concentration of carbamazepine will result in a proportional change in the concentration of the dihydroxy metabolite in plasma.

3.1.8. Correlation between the plasma level of 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine

The relationship between the concentration of 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine shown in figs. 22 and 23 for responders and non-responders, respectively.

The dihydroxy metabolite is formed by an enzymatic

cleavage of the epoxide ring (Faigle 1975). In both responders and non-responders a positive correlation exists between the dihydroxy and epoxy metabolite concentration in plasma; correlation coefficient (r) is 0,78 (P<0,01) and 0,79 (P<0,01) in responders and non-responders, respectively. Since the intercepts of the regression lines do not pass through zero, it is unlikely that a change in the plasma level of the epoxy metabolite will result in a proportional change in the concentration of the dihydroxy metabolite in plasma.

3.1.9. The Spearman Rank Correlation Test

In view of the apparent non-linearity of the correlations, the Spearman rank correlation test was felt to be more appropriate to determine the relationship between carbamazepine and its metabolites and the relationship between the epoxy and dihydroxy metabolites.

	Responders	Non-responders
EPOXY/CBZ	r_s = 0,76, P <0,01	r_s = 0,86, P <0,001
DI-OH/CBZ	r_s = 0,85, P <0,001	r_s = 0,84, P <0,01
DI-OH/EPOXY	r_s = 0,80, P <0,01	r_s = 0,77, P <0,01

(CBZ: carbamazepine; EPOXY: 10,11-epoxy-carbamazepine; DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine; r_s: Spearman rank correlation coefficient.)

This non-parametric test shows unequivocally that there is a positive correlation between the concentration of carbamazepine and the concentration of its metabolites in plasma.

3.1.10. Summary of results

The Mann-Whitney U test was used for all statistical analyses summarized below:

(1) n_1 = 11 non-responders; n_2 = 12 responders.

Parameter	Results
AUC_{CBZ}	n.s.*
$AUC_{DI-OH-CBZ}$	Higher in non-responders (P <0,002)
AUC_{EPOXY}	Higher in non-responders (P <0,02)
Dose (mg/day)	Higher in non-responders (P <0,05)

(2) n_1 = 8 patients on phenobarbitone and/or phenytoin;
n_2 = 8 patients on carbamazepine monotherapy.

$\frac{AUC_{DI-OH}}{AUC_{EPOXY}}$	n.s.*

(3) n_1 = 5 patients on carbamazepine monotherapy (responder group only);
n_2 = 7 patients on phenothiazinesplus carbamazepine (responder group only).

$\frac{AUC_{DI-OH}}{AUC_{EPOXY}}$	n.s.*

* n.s. = not significant at the 5% level.
AUC: Area under the curve
CBZ: Carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine
EPOXY: 10,11-epoxy-carbamazepine

TABLE 11: SIDE-EFFECTS OF CARBAMAZEPINE AND SERUM LEVELS (µg/ml) OF CARBAMAZEPINE AND ITS METABOLITES.

RESPONDERS					NON-RESPONDERS				
INITIALS	SIDE-EFFECTS	CBZ	EPOXY	DI-OH	INITIALS	SIDE-EFFECTS	CBZ	EPOXY	DI-OH
AvA	Nausea (+) Headaches (++)	8,00	1,10	2,80	MF	Drowsiness (++) Headaches (++)	5,10	0,90	4,70
VP	Nausea (+) Headaches (+++)	6,14	0,60	2,10	JN	Nausea (++) Headaches (++)	14,76	3,92	7,24
RI	Drowsiness (+) Headaches (+++)	10,40	2,30	2,70	RA	Drowsiness (++)	7,10	1,30	4,00
MEAN	-	8,18	1,33	2,53	MEAN	-	8,99	2,04	5,31
Patients without side-effects (N = 9)	MEAN	6,11	0,82	2,17	Patients without side-effects (N = 8)	MEAN	8,41	1,73	5,19
	±SD	1,40	0,27	0,65		±SD	2,10	0,51	1,72

Mild (+); moderate (++); severe (+++)
CBZ: Carbamazepine
EPOXY: 10,11-epoxy-carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine
Values are peak serum levels

TABLE 12: INDIVIDUAL VALUES OF AREA UNDER THE CURVE (AUC) (µg/ml.hr) FOR CARBAMAZEPINE AND METABOLITES AND THEIR RESPECTIVE RATIOS IN EPILEPTIC RESPONDERS.

INITIALS	CBZ	EPOXY	DI-OH	EPOXY CBZ	DI-OH CBZ	DI-OH EPOXY
AvA	75,65	10,33	27,48	0,14	0,36	2,66
VP	50,70	5,93	21,25	0,13	0,42	3,58
RI	88,58	24,16	33,90	0,27	0,38	1,40
KS	58,75	11,10	33,35	0,19	0,57	3,00
JA	42,11	5,63	18,12	0,13	0,43	3,22
JT	80,02	11,53	29,34	0,14	0,37	2,54
RT	66,47	9,86	23,75	0,15	0,36	2,40
DN	66,48	9,22	19,58	0,14	0,29	2,12
DH	41,29	5,12	16,12	0,12	0,39	3,15
SS	41,22	5,64	11,85	0,14	0,29	2,10
BR	62,85	4,76	20,68	0,08	0,33	4,34
MA	44,63	8,69	18,45	0,19	0,41	2,12

CBZ: Carbamazepine
EPOXY: 10,11-epoxy-carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine

TABLE 13: INDIVIDUAL VALUES OF AREA UNDER THE CURVE (AUC) (µg/ml.hr) FOR CARBAMAZEPINE AND METABOLITES AND THEIR RESPECTIVE RATIOS IN EPILEPTIC NON-RESPONDERS.

INITIALS	CBZ	EPOXY	DI-OH	EPOXY / CBZ	DI-OH / CBZ	DI-OH / EPOXY
MF	48,46	8,80	46,02	0,18	0,95	5,23
BG	81,50	13,98	47,20	0,17	0,58	3,38
GP	79,03	19,89	63,23	0,25	0,80	3,18
RA	58,66	12,24	39,28	0,21	0,67	3,21
MC	97,24	22,76	71,70	0,23	0,74	3.15
QJ	82,84	20,34	73,36	0,25	0,89	3,60
MB	74,51	16,95	42,94	0,23	0,58	2,53
JN	116,96	35,26	79,28	0,30	0,68	2,25
CC	43,32	9,46	32,01	0,22	0,74	3,38
RC	38,52	12,93	28,18	0,34	0,73	2,18
SH	76,97	13,37	59,03	0,17	0,77	4,41

CBZ: Carbamazepine
EPOXY: 10,11-epoxy-carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine

TABLE 14: MEAN CONCENTRATIONS (µg/ml) OF CARBAMAZEPINE AND METABOLITES IN EPILEPTIC RESPONDERS.

INITIALS	CBZ	EPOXY	DI-OH	PB*	PHT*
AvA	7,58	1,04	2,74	-	-
VP	4,84	0,56	2,13	-	-
RI	8,58	2,39	3,43	-	-
KS	5,87	1,11	3,34	-	10
JA	4,16	0,57	1,81	-	10
JT	7,91	1,15	2,91	-	-
RT	6,47	0,98	2,37	-	-
DN	6,29	0,91	1,94	-	-
DH	4,04	0,51	1,60	-	-
SS	4,05	0,56	1,17	-	-
BR	5,98	0,40	1,95	-	-
MA	4,41	0,87	1,84	-	-

CBZ: Carbamazepine
EPOXY: 10,11-epoxy-carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine
PH: Phenobarbitone
PHT: Phenytoin

* These drugs were assayed by the service laboratory of the Pharmacology Department, University of Cape Town, using the Emit^{R} method for antiepileptic drugs. Values are mean concentrations (µg/ml).

TABLE 15: MEAN CONCENTRATIONS (µg/ml) OF CARBAMAZEPINE AND METABOLITES IN EPILEPTIC NON-RESPONDERS.

INITIALS	CBZ	EPOXY	DI-OH	PB*	PHT*
MF	4,94	0,89	4,59	-	-
BG	8,07	1,38	4,69	-	-
GP	7,77	1,98	6,28	20	-
RA	5,74	1,20	3,93	-	-
MC	9,49	2,10	5,59	-	-
QJ	7,99	1,97	7,28	20	12
MB	7,35	1,66	4,30	-	10
JN	11,59	3,45	7,66	10	-
CC	4,07	0,78	3,20	15	-
RC	3,68	1,27	2,82	-	15
SH	7,67	1,37	6,00	-	-

CBZ: Carbamazepine
EPOXY: 10,11-epoxy-carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine
PB: Phenobarbitone
PHT: Phenytoin

* These drugs were assayed by the service laboratory of the Pharmacology Department, University of Cape Town, using the Emit^{R} method for anti-epileptic drugs. Values are mean concentrations (µg/ml).

TABLE 16: CEREBROSPINAL FLUID (CSF) CONCENTRATIONS OF CARBAMAZEPINE AND METABOLITES.

INITIALS	SERUM CONCENTRATION (µg/ml)			CSF CONCENTRATION (µg/ml)			CSF/SERUM RATIO			$\frac{CSF_{DI-OH}}{CSF_{CBZ}}$	$\frac{CSF_{EPOXY}}{CSF_{CBZ}}$
	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH		
QJ	8,60	1,80	6,50	1,42	1,04	2,21	0,17	0,58	0,34	1,60	0,73
MB	8,06	1,78	4,20	2,00	1,30	1,69	0,25	0,73	0,40	0,85	0,65
MF	5,10	0,90	4,70	1,18	0,66	1,86	0,23	0,73	0,40	1,60	0,56
CC	3,34	0,74	3,22	0,82	0,46	1,10	0,25	0,62	0,36	1,40	0,56
JN	14,50	3,80	7,20	3,16	1,42	3,50	0,22	0,37	0,49	1,10	0,45
GP	8,10	1,95	5,70	2,50	1,43	1,96	0,31	0,73	0,34	0,78	0,57
BG	4,10	0,70	2,00	1,25	0,39	1,10	0,30	0,56	0,55	0,88	0,31
MEAN	7,40	1,67	4,79	1,76	0,96	1,92	0,25	0,62	0,41	1,17	0,55
±SD	3,77	1,08	1,83	0,83	0,45	0,81	0,05	0,13	0,08	0,36	0,14

CBZ: Carbamazepine
EPOXY: 10,11-epoxy-carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine

TABLE 17: DETAILS OF FLUCTUATIONS IN SERUM LEVELS OF CARBAMAZEPINE IN EPILEPTIC RESPONDERS.

INITIALS	DOSE (mg/day)	MINIMUM (Cmin) (µg/ml)	MAXIMUM (Cmax) (µg/ml)	DIFFERENCE (Cmax-Cmin) (µg/ml)	MEAN (µg/ml)	FLUCTUATION (%)
AvA	1 000	7,19	8,14	0,95	7,58	13
VP	800	2,25	6,14	3,89	4,84	80
RI	400	7,12	10,40	3,28	8,58	38
KS	600	5,19	6,48	1,29	5,87	22
JA	400	3,45	4,57	1,12	4,16	27
JT	600	7,19	8,57	1,38	7,91	17
RT	600	5,46	7,36	1,90	6,47	29
DN	400	4,91	7,27	2,36	6,29	38
DH	400	3,11	4,69	1,58	4,04	39
SS	400	3,63	4,95	1,32	4,05	33
BR	1 200	4,45	8,63	4,18	5,98	70
MA	400	3,91	5,08	1,17	4,41	27
MEAN	600	4,82	6,86	2,04	5,85	36
(±) STANDARD DEVIATION	270	1,68	1,86	1,14	1,59	20

TABLE 18: DETAILS OF FLUCTUATIONS IN SERUM LEVELS OF CARBAMAZEPINE IN EPILEPTIC NON-RESPONDERS.

INITIALS	DOSE (mg/day)	MINIMUM (Cmin) (µg/ml)	MAXIMUM (Cmax) (µg/ml)	DIFFERENCE (Cmax-Cmin) (µg/ml)	MEAN (µg/ml)	FLUCTUATION (%)
MF	800	3,98	5,83	1,85	4,94	37
BG	800	5,98	9,40	3,42	8,07	42
GP	800	6,20	9,30	3,10	7,77	40
RA	800	5,0	7,10	2,10	5,74	37
MC	900	7,76	11,0	3,24	9,49	34
QJ	800	6,46	9,98	3,52	7,99	44
MB	800	5,87	9,18	3,31	7,35	45
JN	1 000	9,34	14,76	5,42	11,59	47
CC	600	3,06	5,84	2,78	4,07	68
RC	800	2,23	4,86	2,63	3,68	71
SH	600	6,90	9,31	2,41	7,67	31
MEAN	791	5,71	8,78	3,07	7,12	45
(±) STANDARD DEVIATION	114	2,06	2,81	0,95	2,36	13

TABLE 19: SERUM LEVELS (µg/ml) OF CARBAMAZEPINE AND METABOLITES IN EPILEPTIC RESPONDERS.

	SAMPLING TIME (HOURS)																	
	08.00			10.00			12.00			14.00			16.00			18.00		
INITIALS	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH
AvA	7,19	0,95	2,61	8,00	1,05	2,75	7,33	0,97	2,68	7,37	1,03	2,68	7,46	1,05	2,95	8,14	1,18	2,75
VP	2,25	0,14	1,96	4,29	0,35	2,03	6,14	0,64	2,10	5,77	0,85	2,10	5,49	0,71	2,23	5,07	0,69	2,37
RI	7,12	1,87	2,60	10,04	2,27	2,73	9,32	2,27	2,91	8,80	2,59	3,57	8,34	2,68	4,13	8,46	2,67	4,62
KS	5,19	1,08	3,21	6,25	1,08	3,33	5,89	1,08	3,21	5,75	1,15	3,40	5,65	1,15	3,40	6,48	1,10	3,46
JA	3,45	0,61	1,80	3,77	0,51	1,80	4,57	0,56	1,80	4,36	0,52	1,80	4,43	0,61	1,86	4,40	0,62	1,80
JT	7,19	1,15	2,77	8,29	1,12	2,89	8,57	1,15	2,95	8,11	1,18	3,00	7,62	1,18	3,06	7,65	1,12	2,77
RT	5,71	1,03	2,65	7,36	1,05	2,48	7,19	1,00	2,37	6,65	0,97	2,26	6,45	0,97	2,40	5,46	0,85	2,08
DN	4,91	0,82	2,05	7,27	0,99	2,14	6,53	0,92	1,89	6,94	1,00	2,01	6,03	0,88	1,89	6,03	0,82	1,67
DH	3,11	0,44	1,51	3,59	0,47	1,66	4,51	0,50	1,66	4,69	0,56	1,64	4,25	0,55	1,59	4,10	0,52	1,51
SS	3,63	0,52	1,12	3,83	0,53	1,26	4,95	0,63	1,19	4,11	0,56	1,19	3,99	0,56	1,17	3,83	0,56	1,11
BR	4,45	0,27	1,99	4,53	0,26	1,85	6,23	0,43	2,15	6,04	0,48	2,01	8,63	0,71	2,26	7,54	0,73	2,15
MA	3,91	0,87	1,96	4,19	0,80	1,84	5,08	0,92	1,88	4,84	0,91	1,82	4,07	0,85	1,85	4,36	0,86	1,71

CBZ: Carbamazepine
EPOXY: 10,11-epoxy-carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine

TABLE 20: SERUM LEVELS (µg/ml) OF CARBAMAZEPINE AND METABOLITES IN EPILEPTIC NON-RESPONDERS.

	SAMPLING TIME (HOURS)																	
	08.00			10.00			12.00			14.00			16.00			18.00		
INITIALS	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH	CBZ	EPOXY	DI-OH
MF	4,99	0,97	4,40	3,98	0,77	4,47	4,82	0,85	4,73	5,12	0,92	4,68	4,90	0,90	4,63	5,83	0,95	4,60
BG	5,98	1,00	3,84	7,78	1,08	4,34	7,68	1,18	4,26	9,06	1,68	5,06	8,54	1,74	5,42	9,40	1,62	5,20
GP	7,94	2,06	6,48	7,66	1,93	6,48	9,29	2,21	7,10	8,14	1,95	6,27	7,34	1,95	5,70	6,23	1,75	5,65
RA	5,00	1,06	3,64	5,36	1,04	3,58	7,10	1,34	4,02	6,22	1,34	4,02	5,52	1,28	4,08	5,26	1,18	4,24
MC	7,76	1,74	5,48	11,0	2,28	6,52	10,82	2,54	7,68	9,42	2,34	7,12	9,06	2,26	7,86	8,88	2,18	7,86
QJ	6,54	1,66	6,14	8,60	1,84	6,50	9,98	2,32	6,92	9,02	2,36	7,92	7,32	1,98	8,34	6,46	1,68	7,86
MB	6,66	1,57	4,26	9,18	1,86	4,43	8,06	1,78	4,20	7,32	1,74	4,40	5,87	1,48	4,11	6,99	1,54	4,40
JN	9,60	3,16	7,24	14,76	3,78	7,66	13,18	3,94	8,34	9,34	3,92	8,02	10,10	2,94	8,00	12,60	2,94	8,00
CC	3,82	0,98	2,96	3,34	0,74	3,22	5,84	1,10	3,46	4,30	0,96	3,26	4,02	0,96	3,08	4,50	0,96	3,01
RC	2,23	0,88	2,10	3,05	0,92	2,25	4,86	1,41	2,56	4,54	1,54	3,02	3,99	1,42	3,38	3,41	1,47	3,66
SH	7,18	1,52	6,96	7,04	1,16	5,44	9,31	1,60	6,18	7,91	1,38	5,56	6,90	1,19	5,98	7,47	1,19	5,75

CBZ: Carbamazepine
EPOXY: 10,11-epoxy-carbamazepine
DI-OH: 10,11-dihydro-10,11-dihydroxy-carbamazepine

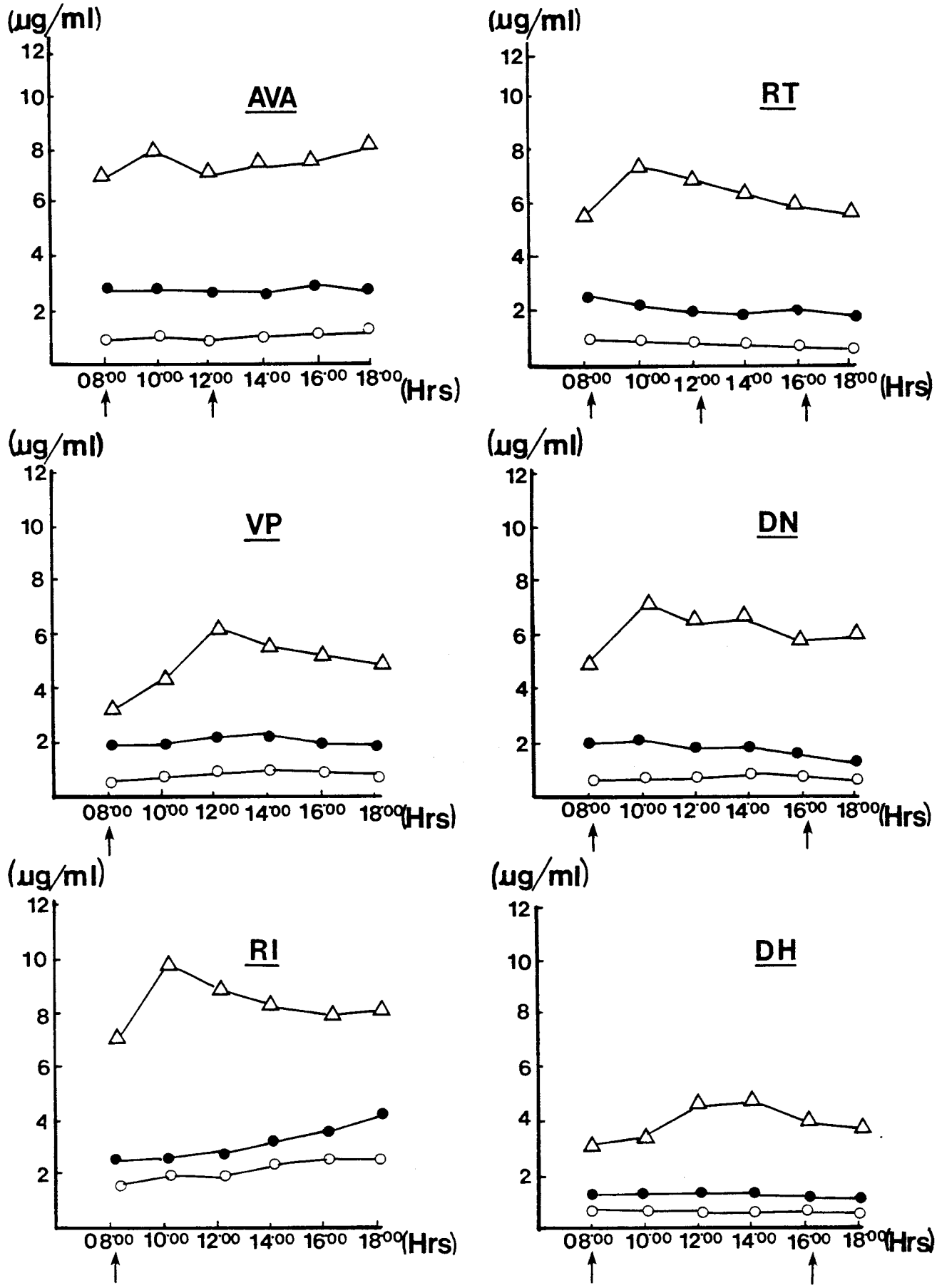


Fig. 14: Diurnal profiles of carbamazepine and metabolites in epileptic responders. (△—△Carbamazepine; ○—○ 10,11-Epoxy- Carbamazepine; ●—● 10,11-Dihydro-10,11-Dihydroxy-Carbamazepine; ——→Dose)

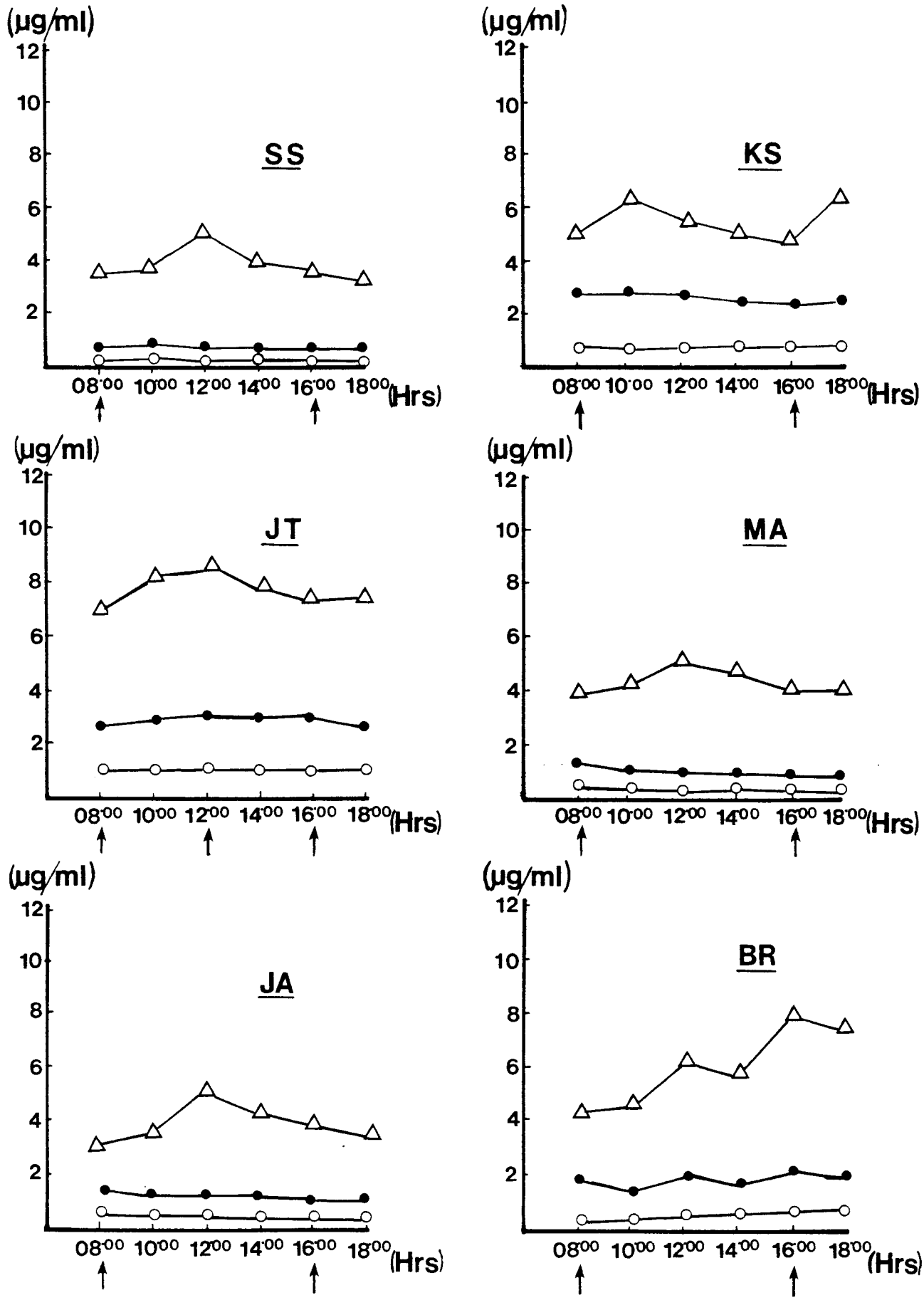


Fig. 15: Diurnal profiles of carbamazepine and metabolites in epileptic responders. (△——△ Carbamazepine; ○——○ 10,11-Epoxy-Carbamazepine; ●——● 10,11-Dihydro-10,11-Dihydroxy-Carbamazepine; ——→ Dose)

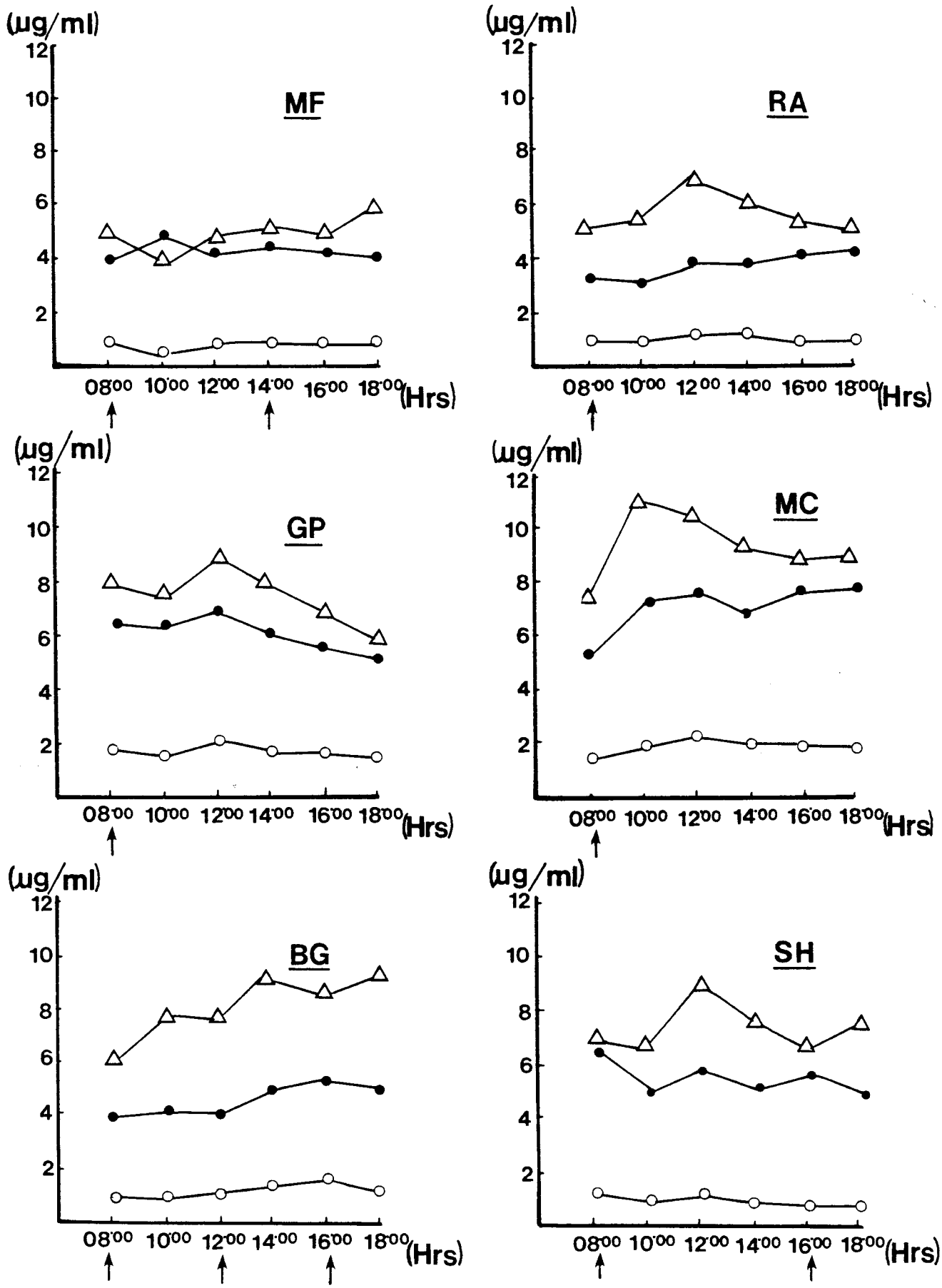


Fig. 16: Diurnal profiles of carbamazepine and metabolites in epileptic non-responders. (△——△ Carbamazepine; ○——○ 10,11-Epoxy-Carbamazepine; ●——● 10,11-Dihydro-10,11-Dihydroxy-Carbamazepine; ·——→ Dose)

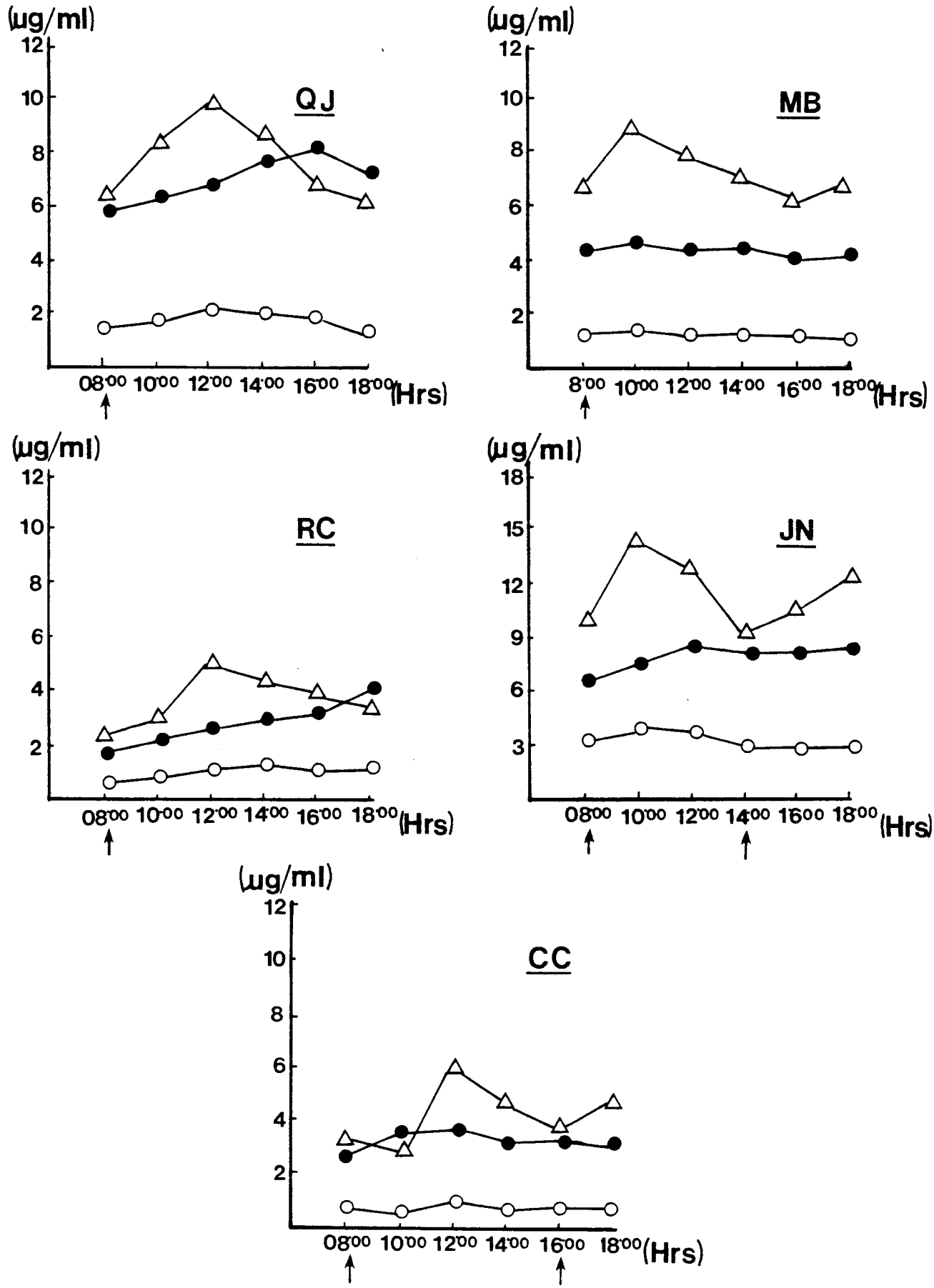


Fig. 17: Diurnal profiles of carbamazepine and metabolites in epileptic non-responders. (△——△ Carbamazepine; ○——○ 10,11-Epoxy-Carbamazepine; ●——● 10,11-Dihydro-10,11-Dihydroxy-Carbamazepine; ——→ Dose)

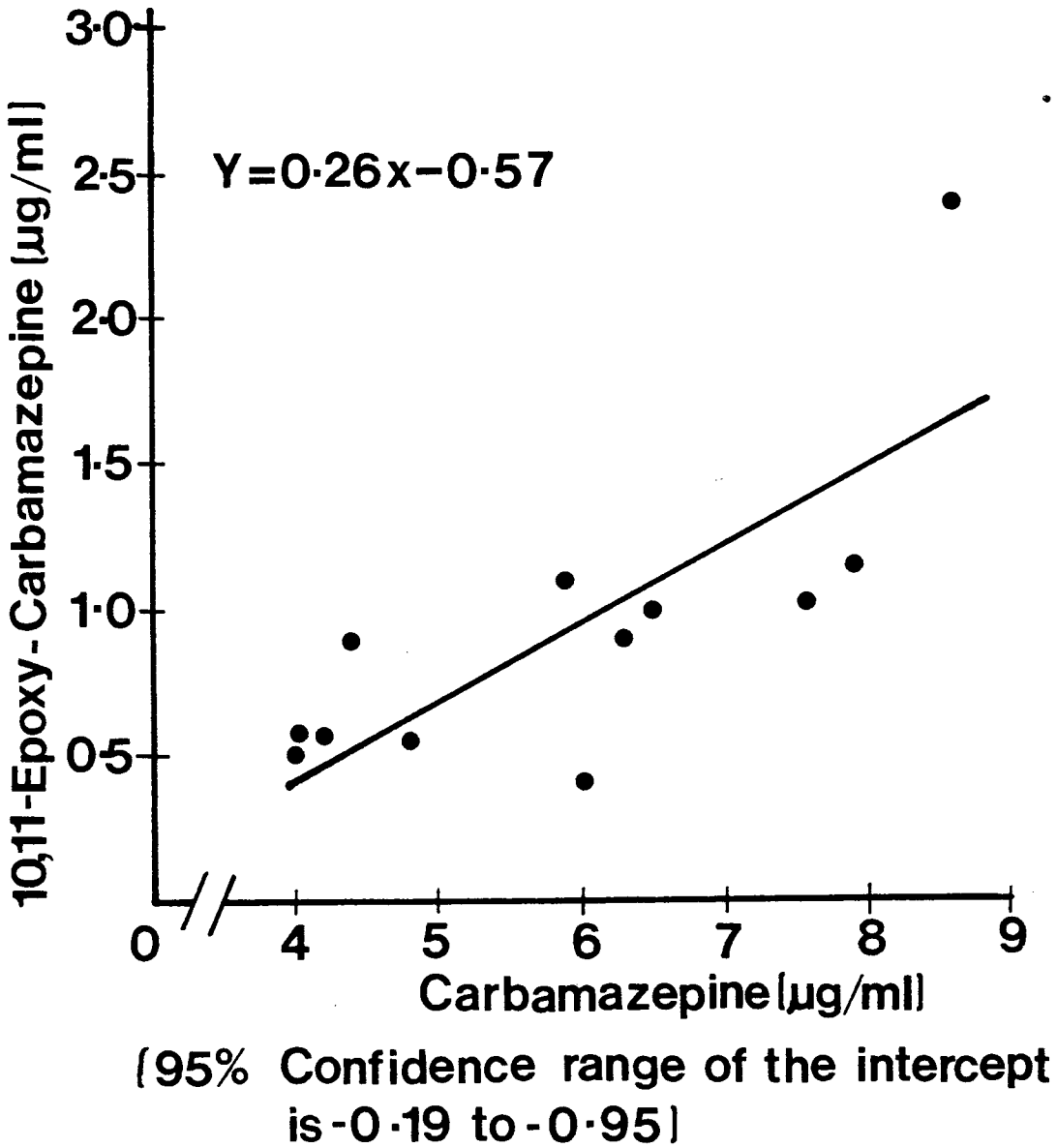


Fig. 18: Linear regression of mean plasma concentrations of 10,11-epoxy-carbamazepine upon carbamazepine in epileptic responders. Correlation coefficient, $r = 0.76$, $P < 0.01$.

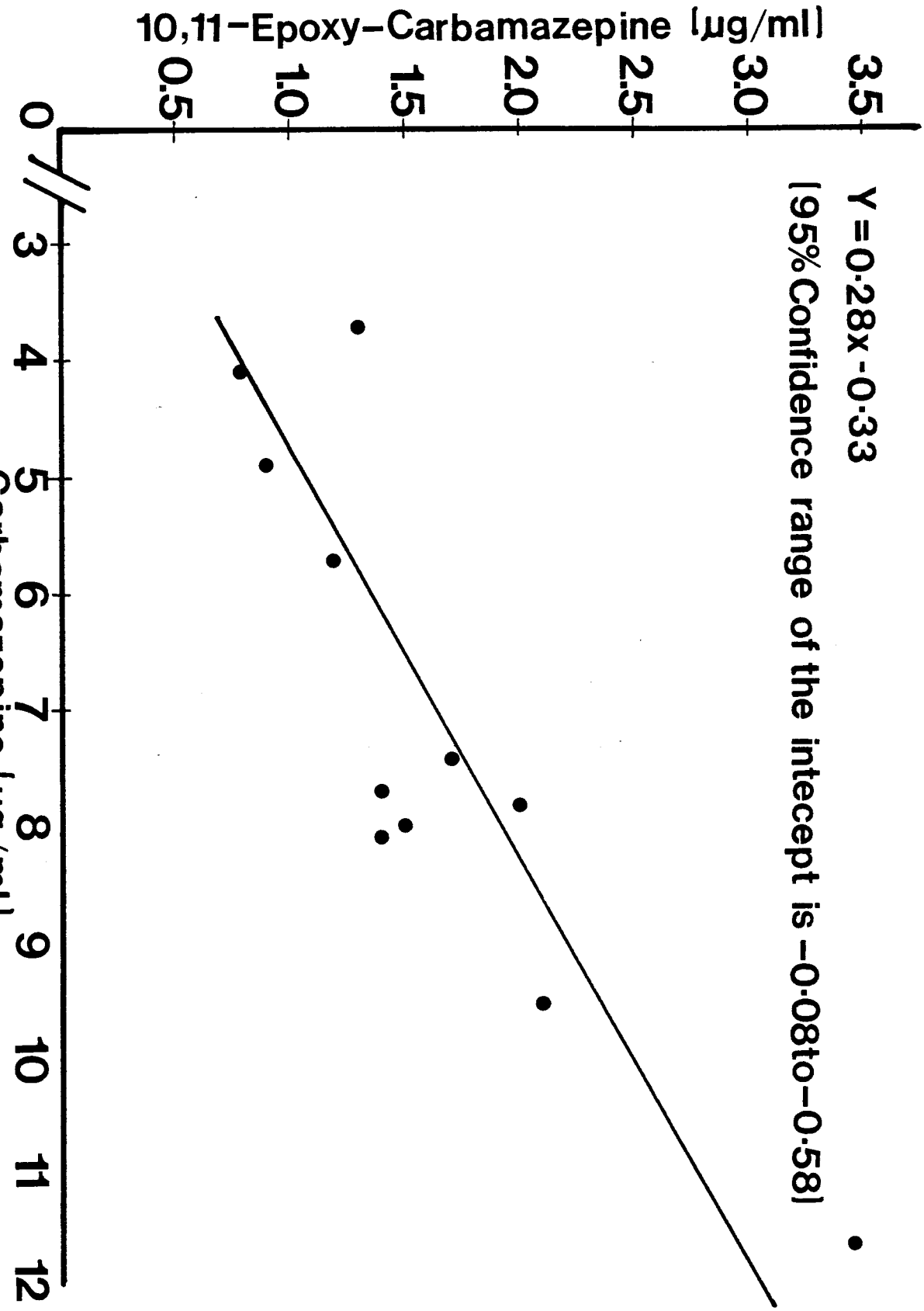


Fig. 19: Linear regression of mean plasma concentrations of 10,11-epoxy-carbamazepine upon carbamazepine in epileptic non-responders. Correlation coefficient, $r = 0.88$, $P < 0.001$.

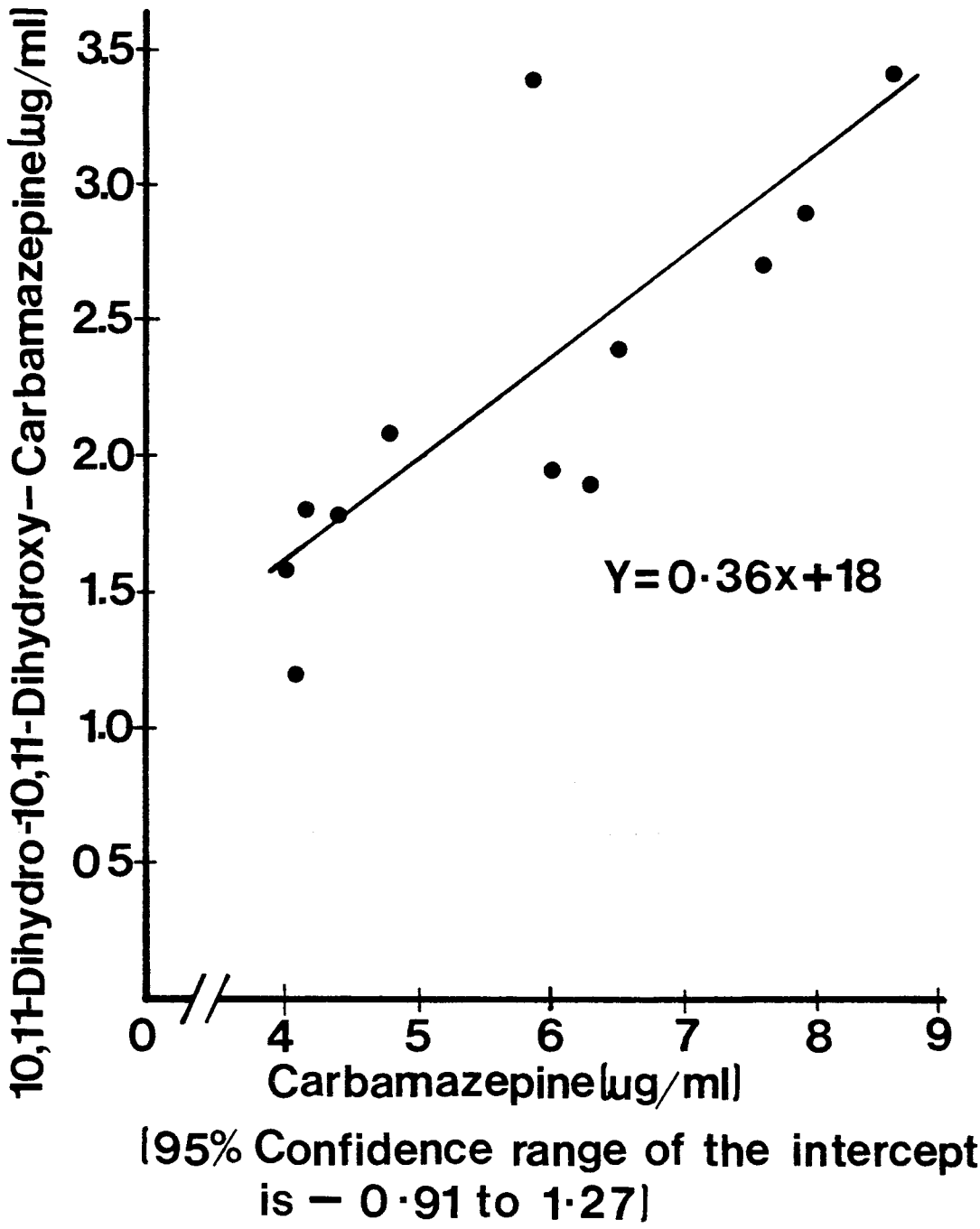


Fig. 20: Linear regression of mean plasma concentration of 10,11-dihydro-10,11-dihydroxy-carbamazepine upon carbamazepine in epileptic responders. Correlation co-efficient, r = 0.81, $P < 0.01$.

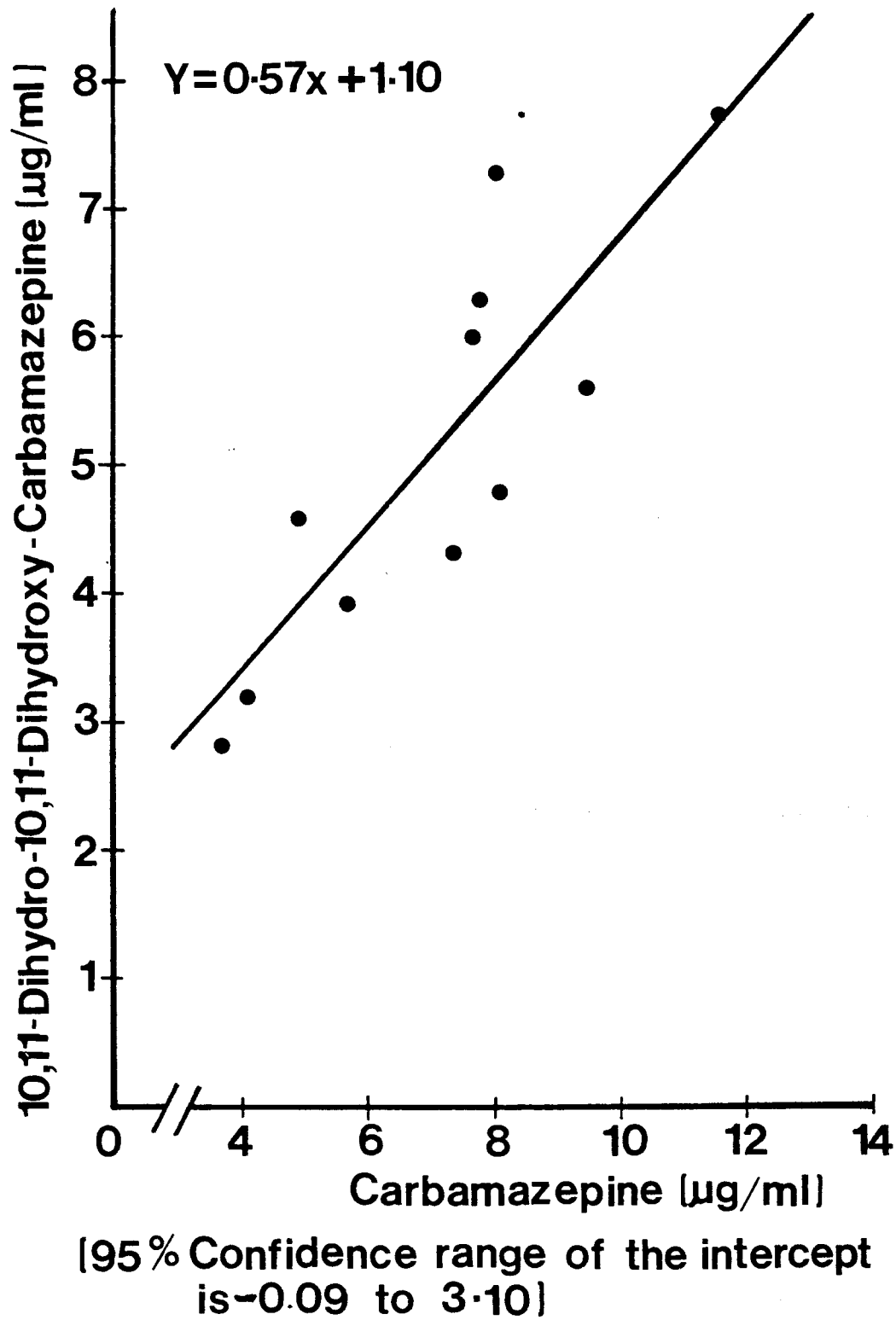


Fig. 21: Linear regression of mean plasma concentrations of 10,11-dihydro-10,11-dihydroxy-carbamazepine upon carbamazepine in epileptic non-responders. Correlation coefficient, $r = 0.85$, $P < 0.001$.

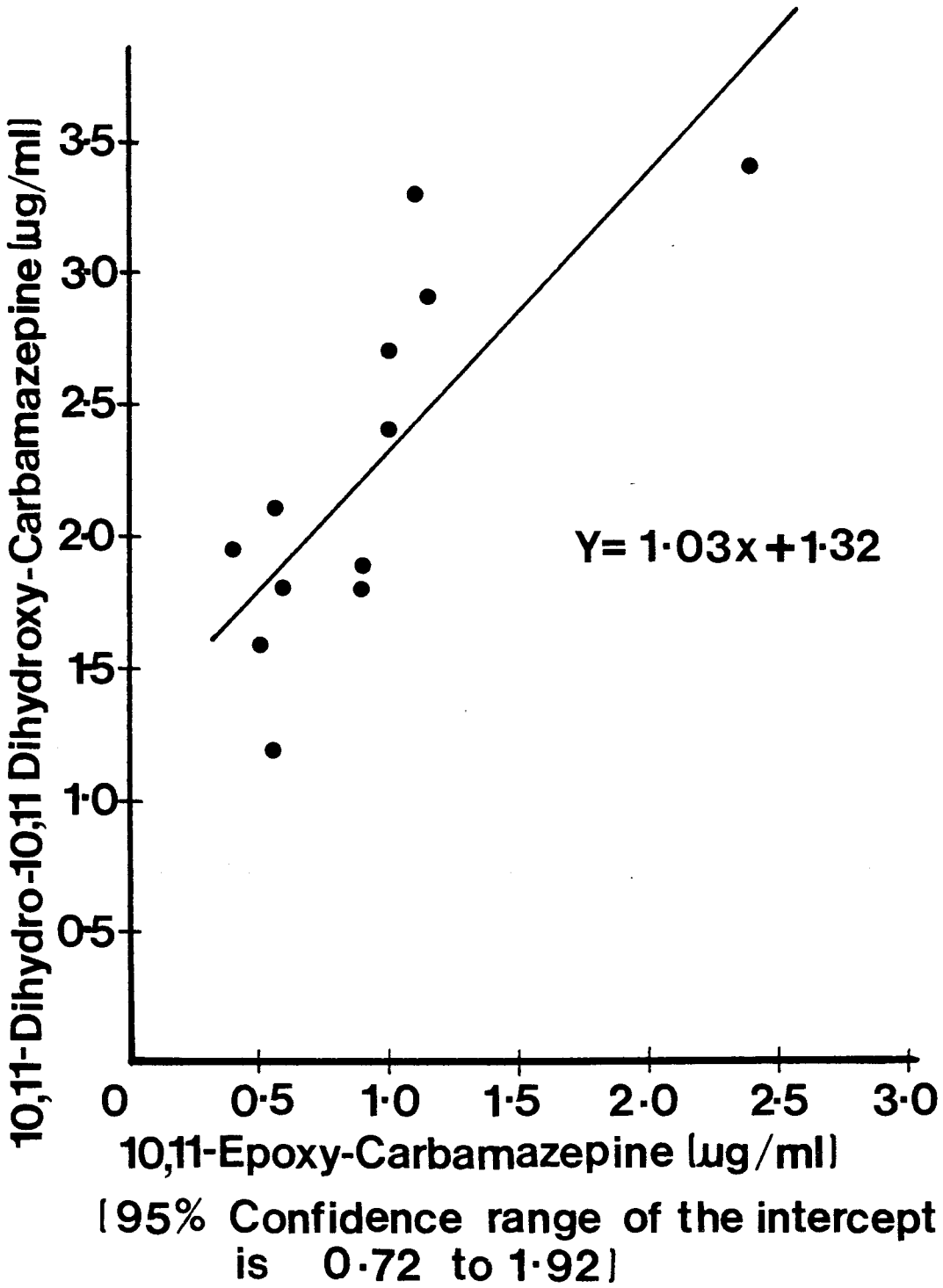


Fig. 22: Linear regression of mean plasma concentrations of 10,11-dihydro-10,11-dihydroxy-carbamazepine upon 10,11-epoxy-carbamazepine in epileptic responders. Correlation coefficient, $r = 0.78$, $P < 0.01$.

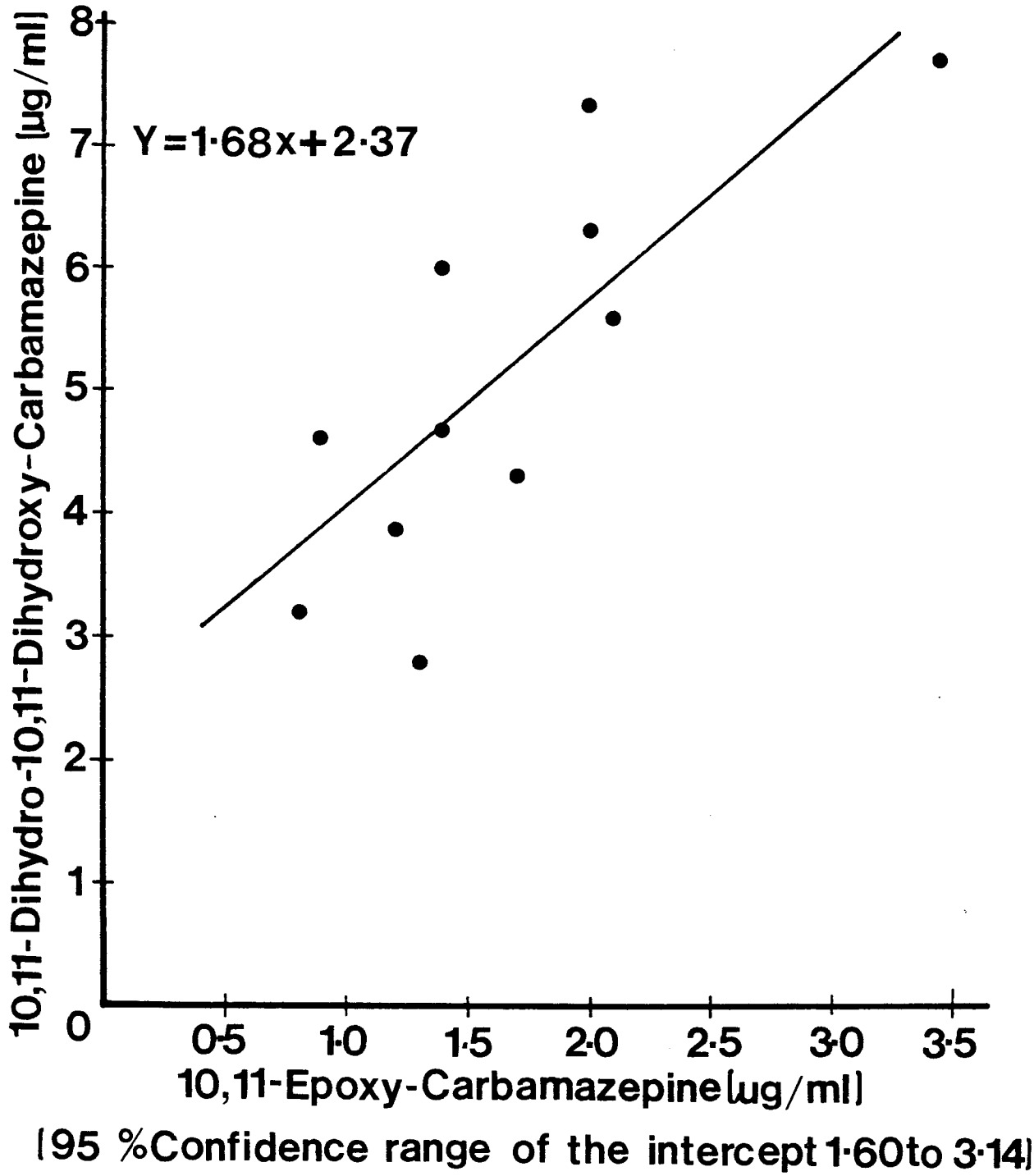


Fig. 23: Linear regression of mean plasma concentrations of 10,11-dihydro-10,11-dihydroxy-carbamazepine upon 10,11-epoxy-carbamazepine in epileptic non-responders. Correlation coefficient, $r = 0.79$, $P < 0.01$.

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

CHAPTER FOUR

4.1. Discussion

4.1.1. Side-effects

Carbamazepine has been reported to cause a wide variety of side-effects such as drowsiness, ataxia, nausea and vomiting, skin rashes, depression and confusion, and various haematological abnormalities (review by Gayford and Redpath 1969).

However, it must be stressed that these side-effects are often manifested at the start of therapy and usually disappear on continuation of the treatment.

Hoppener et al (1980) monitored carbamazepine serum levels between 8 a.m. and 6 p.m. in 43 epileptic patients. A threshold level of 8 μg/ml was reported above which intermittent side-effects - diplopia, drowsiness and headaches - were likely to occur. This study confirmed earlier reports by Parsonage (1972), Meinardi (1972) and Schneider (1975) who suggested that the clinician could prevent serious side-effects by ensuring that the carbamazepine serum level is below 8 to 9 μg/ml.

It is unfortunate, however, that the main metabolites of carbamazepine, viz. 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine were not monitored in these studies. Both metabolites are only approximately 50% bound to plasma proteins (based on simultaneous cerebrospinal fluid/plasma level measurements, Schneider and Berenguer 1975) therefore quite a large fraction of these metabolites can

enter the central nervous system and they may in fact play a role in the manifestations of side-effects.

In this study 6 out of the 23 patients complained of side-effects; nausea and headaches were the most frequently mentioned complaint (Table 11). In the 6 patients, side-effects occurred with a peak in the serum level of carbamazepine (figs. 14 to 17).

In the responder group, the mean peak serum level of carbamazepine in the patients with side-effects was 8,18 µg/ml compared with 6,11 µg/ml in the patients without side-effects. In the non-responder group, the mean peak serum level of carbamazepine in the patients with side-effects was 8,99 µg/ml compared with 8,41 µg/ml in the patients without side-effects. The point must be made here that patients in the non-responder group were apparently too concerned with the uncontrolled nature of their seizures to complain about side-effects. The randomization test for two independent samples showed, however, that there was no significant difference between the mean peak levels of carbamazepine in patients, both with or without side-effects, in either the responder or non-responder groups. Therefore, a threshold level above which side-effects are likely to occur could not be defined.

In the case of 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine, again statistical analysis showed that there was no significant difference in the serum levels of these metabolites in patients, both with or without side-effects. Thus, no definite conclusion could be reached as to whether the metabolites play a role in the manifestation of side-effects.

For the three patients in the responder group, reduction in the morning dose of carbamazepine brought about a disappearance of side-effects with still very good control of seizures. As regards the three patients in the non-responder group, the problem was more complex because a balance had to be struck between seizure control, such as it was, and the incidence of side-effects. Patients MF and JN had their drug regimen changed, while for patient RA, the neurologist decided that the side-effects were not severe enough to warrant a reduction in dosage (see Appendix B, Case Studies, for more details.)

It is interesting to note that these six patients who complained of side-effects had been taking carbamazepine for at least a year. The side-effects were not spontaneously mentioned and would have escaped notice if attention had not been focussed on them. The patients believed that drowsiness, nausea and headaches were connected with their epilepsy and they accepted it, more so because in the past very little attention had been paid to their complaints. The clinician must therefore focus his attention on possible side-effects occurring with a peak in the level of carbamazepine and make specific enquiries about these reactions and monitor serum levels at intervals during the day if there is any doubt about toxicity.

4.1.2. Role of the metabolites in the therapeutic efficacy of carbamazepine

The chemical and physical properties of carbamazepine are such that it can be classified as a neutral, lipophilic compound (Faigle et al 1976). The lipid solubility is attributable to its tricyclic framework, and its neutral character can be ascribed to the fact that the nitrogen atoms in the azepine ring and carbamoyl side chain are part of a urea moiety (fig. 1).

The body possesses no mechanism by which exogenous lipophilic substances, including especially those of neutral character, can be excreted in unchanged form (Weiner 1967). In the case of carbamazepine, it has been found in man that only about 1% of the drug is excreted unchanged in the urine and 70% in the form of metabolites; the main one being 10,11-dihydro-10,11-dihydroxy carbamazepine (Faigle and Feldman 1975).

As a result of this rather extensive metabolism, attention was focussed on the possibility that 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine could perhaps play a role in the overall anticonvulsant activity of carbamazepine in man.

Carbamazepine and metabolites were therefore administered as single oral doses to mice and rats and tested for anticonvulsant activity (Frigerio and Morselli 1975; Schmutz et al (1979). From the ED-50, the dose required to protect 50% of the animals against convulsions induced by electric shock or cardiazol, it was found that only 10,11-epoxy-carbamazepine could significantly add to the anti-epileptic effect of

carbamazepine in patients.

In the literature, only one report is to be found in which the correlation between seizure frequency and plasma metabolite concentrations has been investigated. Dam et al (1977) studied 132 epileptic patients who had been treated for 1 to 8 years with carbamazepine either alone or in combination with other anticonvulsants. Apart from carbamazepine, only 10,11-epoxy-carbamazepine was monitored. The authors anticipated that if 10,11-epoxy-carbamazepine had an anti-epileptic effect of its own in man, then patients with high plasma level of this metabolite would have better seizure control. However, it was found that patients who had relatively higher levels of 10,11-epoxy-carbamazepine did not in fact have better seizure control.

In agreement with the above study, I found that patients in the non-responder group had higher serum levels of 10,11-epoxy-carbamazepine relative to those patients in the responder group ($P < 0,02$). The higher epoxy metabolite levels, in the non-responders, were associated with a higher relative serum level of 10,11-dihydro-10,11-dihydroxy-carbamazepine in these patients ($P < 0,002$).

This suggests, therefore, that the metabolites of carbamazepine are inactive in man, or that 10,11-epoxy-carbamazepine is active (as in rats; Frigerio and Morselli 1975) but that any likely therapeutic effect is counteracted by the relatively large concentration of 10,11-dihydro-10,11-dihydroxy-carbamazepine which has been shown to be inactive (Schmutz et al 1979).

4.1.3. Why no response to carbamazepine?

In the management of epilepsy, it is now accepted that patients must have an optimal level of anticonvulsant drug in their plasma in order to achieve control of seizures. This optimum concentration can be defined as that concentration of drug which brings about maximal control of seizures with the minimum of side-effects.

For a drug like carbamazepine, it is better to speak of optimal levels because there is some difference of opinion about the therapeutic range (as is commonly the case when the range is based on data from several small trials, not always properly controlled analytically and statistically). A reasonable guess is that the optimal level can be as low as 2 μg/ml to as high as 12 μg/ml (refer Chapter One, 1.3.4).

In the various clinical trials reported in the literature, it has often been pointed out that it is not understood why certain patients with optimal levels of carbamazepine in plasma fail to respond to therapy. For example, Eichelbaum et al (1976) reported that in 13 patients suffering from partial complex seizures, carbamazepine levels of 5 μg/ml did not produce any improvement in the frequency of partial or generalised seizures. Increasing the dose in 5 of those patients produced plasma concentrations of 7 to 8 μg/ml; there was no improvement in seizure frequency and side-effects were seen. More recently, Strandjord and Johannessen (1980) reported that out of 62 patients taking part in a study to evaluate the efficacy of carbamazepine, as sole drug in the treatment of simple and complex partial

seizures, 20% failed to respond to therapy even with optimal levels of carbamazepine (5 to 8 µg/ml) and 10,11-epoxy-carbamazepine (0,4 to 1,2 µg/ml) in plasma.

In agreement with these studies, I found that there was no statistically significant difference in the area under the plasma concentration/time curve (AUC) for carbamazepine, between responders and non-responders. The AUC gives the overall concentration of carbamazepine in the plasma of the individual patients of the two groups. Moreover, the mean concentration of carbamazepine in the responder group ranged from 4,82 to 6,86 µg/ml (Table 17) and in the non-responder group the range of mean carbamazepine concentrations was 5,71 to 8,78 µg/ml (Table 18).

It is a well-known clinical impression that partial complex seizures are more resistant to anti-epileptic treatment than generalised (tonic-clonic) seizures. In this study, the patient population in the responder group consisted of six patients suffering from partial complex seizures and six patients with generalised (tonic-clonic) seizures. In the non-responder group, five patients had partial complex seizures and six patients suffered from generalised (tonic-clonic) seizures. Therefore, it can be seen that there was no bias, in the type of epilepsy, of the patient population of the two groups.

The medication charts of the two patient groups are quite different (Tables 3 and 4). Six patients in the responder group were taking phenothiazines (chlorpromazine, fluphenazine, and clozapine). However, these drugs do not

possess anticonvulsive properties and in fact it has been shown that grand mal or focal seizures may occur on phenothiazines, especially in patients with a history of convulsive disorder (Simpson and Cooper 1978).

Moreover, patients in the non-responder group did not differ markedly from the responders with respect to the cause of epilepsy (see Appendices A and B). The mean duration of epilepsy in the responders and non-responders was 12,3 years.

The question, therefore, is how do we explain non-response to carbamazepine in the presence of optimal levels of the drug in plasma?

Statistical analysis of available data reveals that the only difference between the patients in the responder and non-responder groups, is the fact that the non-responding patients, although they have optimal levels of carbamazepine in plasma, also have in addition a higher concentration of 10,11-epoxy-carbamazepine ($P < 0,02$) and 10,11-dihydro-10,11-dihydroxy-carbamazepine ($P < 0,002$).

Cerebrospinal fluid (CSF) was obtained from seven patients of the non-responder group. On ethical grounds, it was not possible to obtain CSF from responders, so a statistical comparison between the two groups could not be made. However, the mean (±SD) ratio of 10,11-epoxy-carbamazepine to carbamazepine in the non-responders was 0,55±0,14 while the mean (±SD) ratio of 10,11-dihydro-10,11-dihydroxy-carbamazepine to carbamazepine was as high as 1,17±0,36 (Table 16).

From the fact that the non-responding patients had a

much higher concentration of 10,11-dihydro-10,11-dihydroxy-carbamazepine in plasma and CSF, it appears that this metabolite is as inactive in man as in animals (Schmutz et al 1979).

This has led me to put forward the hypothesis that certain patients fail to respond to carbamazepine, even with optimal levels of the drug in plasma, due to competition at the carbamazepine "receptor" sites in the brain by 10,11-dihydro-10,11-dihydroxy-carbamazepine.

It must be pointed out that no actual receptor has been isolated for carbamazepine. The term "receptor" is being used to describe any system in the brain, with which carbamazepine is interacting, in order to produce its characteristic pharmacological effect, that is, preventing the spread of an epileptic discharge.

It has been shown that carbamazepine may exert its therapeutic effect by blocking the accumulation of cyclic 3',5'-adenosine monophosphate (cAMP). Cyclic AMP may play a vital role in epileptogenesis (Lewin and Bleck 1977). Koella et al (1976) showed that carbamazepine can block hippocampal after-discharge, thus explaining the possible benefit of the drug in temporal lobe seizures. The nucleus ventralis anterior of the thalamus has been implicated in the generation and spread of a seizure discharge. Carbamazepine has been shown to have a specific inhibitory action on the transmission of impulses at this particular site in the brain (Julien & Hollister 1975) Carbamazepine has also the ability to depress the excitability

of membranes, thus raising the threshold for an epileptic discharge (Schauf et al 1974).

At all these possible sites of action of carbamazepine, competition by the inactive 10,11-dihydro-10,11-dihydroxy-carbamazepine will result in a reduced therapeutic effect of the drug.

Patients in the responder group had very good control of their seizures (see Appendix A) with low levels of 10,11-dihydro-10,11-dihydroxy-carbamazepine in their plasma. Therefore, it may be postulated that the binding of this metabolite, at the possible sites of action of carbamazepine, occurs on a competitive basis and that patients not responding to therapy, with high plasma metabolite levels, may have the frequency of their seizures improved by blocking the production of 10,11-dihydro-10,11-dihydroxy-carbamazepine.

4.1.4. Why high metabolite concentrations in non-responders?

No statistical difference could be demonstrated between the concentration of carbamazepine in the responder and non-responder groups. Yet the non-responders had a higher concentration of the metabolites present in their plasma. The daily oral intake of carbamazepine (mg/day) was found to be statistically higher for patients in the non-responder group compared with patients in the responder group ($P < 0,05$). Therefore it is not unlikely that the patients in the non-responder group were metabolising carbamazepine faster than the patients in the responder group, thus obtaining similar carbamazepine levels in plasma but higher levels of 10,11-

epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine.

Six patients in the non-responder group were taking phenobarbitone and/or phenytoin as concurrent medication with carbamazepine. It could therefore be argued that the high metabolite concentrations in the non-responders could have been due to enzyme induction by these drugs, An interaction demonstrated by Christianssen and Dam (1973) and confirmed by Johannessen and Strandjord (1975) and Schneider (1975). However, no significant difference in absolute concentration of the metabolite was found in patients on polypharmacy and patients that were on carbamazepine monotherapy in that group. This suggests that these patients were already in an auto-induced state and their metabolism was not influenced by either phenobarbitone or phenytoin.

The phenothiazines are hydroxylated by the cytochrome P-450 system of the liver, which activates molecular oxygen for the oxidation of these lipid soluble substances. Drugs that are metabolised by the same oxidizing system of cytochrome P-450 will compete with each other for metabolism. For example, thioridazine has been shown to block the metabolism of phenytoin (Vincent 1980).

The formation of 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine is catalysed by the enzymes mono-oxygenase and epoxide hydrase, respectively. These enzymes form an intricate part of the cytochrome P-450 oxidizing system of the liver. It is possible, therefore, that concurrent administration of phenothiazines could well have an inhibitory effect on the metabolism of carbamazepine. However, in this study, differences in serum concentration of

10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine between the 7 patients on phenothiazines and the 5 on carbamazepine monotherapy, could not be demonstrated statistically.

10,11-dihydro-10,11-dihydroxy-carbamazepine is the main metabolite isolated from urine (approximately 35% of the dose), one-third of which is in the form of a glucuronide conjugate (Richter et al 1978). The high concentration of this metabolite present in the non-responders could therefore have been partly due to a clearance problem in these individuals.

In the elimination of a metabolite from the body, there are two processes involved: (a) the rate of formation, and (b) the rate of elimination of the metabolite. The general assumption is that the rate of elimination is always greater than the rate of formation since metabolites are considered to be polar compounds and hence readily eliminated from the body. This assumption may be true when polar conjugates such as glucuronides, sulphates and glycine conjugates are the major metabolites formed. However, the assumption need not be true when biotransformation results in acetylation or oxidation (Gibaldi and Perrier 1975).

Since the serum urea and creatinine were within normal limits in both responders and non-responders (see Appendix C) it may be assumed that the rate of elimination of 10,11-dihydro-10,11-dihydroxy-carbamazepine was the same in both groups of patients. The high levels of the dihydroxy metabolite in the non-responders could therefore perhaps be explained by a greater rate of formation.

There was a greater diurnal fluctuation (mean±SD) in the serum level of carbamazepine in the non-responders (45±13%) than in responders (36±20%). This suggests a shorter half-life of the drug in the non-responders as a result of a greater rate of metabolism. However, the time to reach peak concentrations and the peak levels attained showed wide inter-individual variations in both responders and non-responders and these factors may also have contributed to the diurnal fluctuations in serum levels.

The mixed function oxidases (cytochrome P-450), which includes the enzymes mono-oxygenase and epoxide hydrase, are subject to nutritional and genetic control, so the amounts of epoxides and diols produced will vary from individual to individual (Garner 1976). Moreover, it has been suggested on the basis of results obtained from controlled studies in twins, that genetic factors are more important than environmental factors in determining the rates of drug metabolism in man (Vesell 1972). Identical twins, even when living in different environments, still metabolise drugs at the same rate, demonstrating genetic control of drug metabolism. Isoniazid, which is used in the chemotherapy of tuberculosis, is a classic drug whose metabolism has been shown to be under genetic control (La Du 1972). Many other examples exist in the literature.

It is conceivable therefore that a sub-population of patients exists that are fast metabolisers of carbamazepine, and these are the patients that fail to respond to carbamazepine even with therapeutic levels of the drug in plasma.

Cigarette smoke contains the polycyclic hydrocarbon, benzo(a)pyrene, and this compound has been shown to have the ability to induce liver enzymes (Nebert et al 1974). For example, peak plasma levels of phenacetin at two hours were significantly lower in average smokers than in non-smokers after a 900 mg dose. Ratio of plasma metabolites as total N-acetyl-p-aminophenol (paracetamol) and phenacetin were higher in smokers than in non-smokers

Although the effect of smoking is apparently a selective process, at present it is not possible to predict which drugs will be affected. Nevertheless, it is now thought that the smoking habit should be considered as one of the primary sources of drug interactions in man (Park and Breckenridge 1981).

In this study there were no unusually heavy smoking habits in patients of the non-responder group compared with patients in the responder group and therefore it is unlikely that cigarette smoking could be the reason for high metabolite levels in non-responders.

If the ratio of 10,11-dihydro-10,11-dihydroxy-carbamazepine to carbamazepine is measured at 6 hours after a dose, and the data analysed using the Student's t distribution, it is found that there is a 95% chance that a patient with a ratio of greater than 0,55 will be a non-responder.

4.1.5. Clinical implications

Clinicians with a good understanding of clinical pharmacology tend to adjust the dose of a drug to the needs of the individual patient. Fast metabolisers of the drug will have their dose increased in order to achieve better seizure control, whereas slow metabolisers will have their dose reduced in order to avoid toxic effects.

In the case of carbamazepine, this study presents results which suggest that increasing the dose in fast metabolisers of the drug, may have virtually no beneficial therapeutic effect. Like most drugs, carbamazepine is metabolised by a first order process (Perucca et al 1980). The metabolism of carbamazepine is peculiar, however, in that the two metabolites considered are lipophilic and not readily excreted so that they may persist in the blood and presumably also in the fatty tissues of the central nervous system. Therefore increasing the dose in a fast metaboliser may produce greater concentrations of metabolites in the body. If 10,11-dihydro-10,11-dihydroxy-carbamazepine does indeed interfere at the sites of action of carbamazepine in the brain, then no improvement in seizure control will be expected.

All patients on carbamazepine should therefore have both 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine levels monitored. Patients who are found to have high levels of metabolites and are also non-responders should either have the drug withdrawn or in some way have the metabolism blocked by administration of an oxidative enzyme inhibitor.

4.2. Conclusions

(1) The investigational design used in this study has shown that 10,11-epoxy-carbamazepine and 10,11-dihydro-10,11-dihydroxy-carbamazepine do not play any apparent role in the overall therapeutic efficacy of carbamazepine nor in the manifestation of side-effects.

(2) Non-response to carbamazepine, despite optimal levels of the drug in plasma, is most probably due to competition by 10,11-dihydro-10,11-dihydroxy-carbamazepine at the sites of action of the drug in the brain.

4.3. Suggestions for further work

This study has shown that there is a possibility that the metabolite 10,11-dihydro-10,11-dihydroxy-carbamazepine competitively inhibits the sites of action of carbamazepine in the brain. In order to prove or disprove this hypothesis, further investigations must be carried out.

(1) Animal studies

Seizures to be induced in rats or mice by means of an alternating current (50-100 volts, 50 c.p.s., for 0,63 seconds, applied through corneal electrodes). Carbamazepine then to be injected intrathecally, one hour prior to the electric shock, in increasing doses and the ED_{50} value to be estimated, i.e. the dose required to protect 50% of the animals against tonic extension seizures of the hind limbs. (Intrathecal injection is necessary in order to avoid metabolism.)

Two hours after the injection of the ED_{50} dose of carbamazepine, increasing doses of the metabolite 10,11-dihydro-10,11-dihydroxy-carbamazepine to be injected. If the incidence of tonic convulsions of the hind limbs increases, the metabolite might indeed be competing with the action of the parent substance in the brain.

(2) In vitro studies

From its structure it appears that carbamazepine may have a certain degree of affinity for the dopamine or 5-hydroxy-trytamine (5-HT) receptor.

A radiotracer assay, using a radio-labelled form of a compound known to bind to either the dopamine or 5-HT receptor with high affinity, should therefore be set up.

The nonradioactive form of carbamazepine then to be added in increasing concentrations and the percentage binding of radio-labelled compound against concentration of carbamazepine plotted. This gives the relative affinity of the drug for the receptor. The same procedure then to be repeated with the nonradioactive form of the metabolite, 10,11-dihydro-10,11-dihydroxy-carbamazepine. If similar curves are obtained, then the possibility exists that the metabolite competes with the drug at that particular receptor site. However, a negative result will not mean that in vivo competition by the metabolite at the sites of action of the drug does not take place.

(3) Clinical studies

Drugs like cimetidine and propoxyphene have been shown to have the ability to inhibit the metabolism of carbamazepine (Dam and Christiansen 1977, Telerman-Toppet et al 1981).

An experimental study could be set up to investigate whether inhibition of metabolism by the above drugs, in a non-responder, results in an improvement in seizure control.

(4) Carbamazepine polymorphism

The possibility exists that the metabolism of carbamazepine is under genetic control. In order to examine this,

blood concentrations of carbamazepine and the dihydroxy metabolite should be measured at 6 hours after a standard dose in a large number of subjects. If a bimodal distribution is then found, slow and fast metabolisers of the drug probably exist. However, since carbamazepine induces its own metabolism, subjects should be in steady state conditions before blood sampling.

APPENDICES

AND

REFERENCES

APPENDIX A

CASE STUDIES OF PATIENTS IN THE RESPONDER GROUP:

1. Patient: AvA

   Sex: Female

   Age: 20

   Race: White

   Folder no.: 22001327

History:

Onset of epilepsy: This patient has been suffering from idiopathic epilepsy for the past nine years.

Seizure pattern: Grand mal attacks with a photosensitive component.

Drug therapy: She was started on phenytoin which made her hirsute; phenobarbitone made her aggressive; clonazepam gave her rhisus sardonicus; sodium valproate gave her a persistent vaginal discharge. Her seizures were poorly controlled on these drugs.

In 1978, carbamazepine 200 mg three times a day was started which was gradually increased to 1 000 mg per day; this resulted in relatively good control. However, she has been complaining of headaches and nausea. On reducing the morning dose from 400 mg to 200 mg, these side-effects have disappeared with still very good control of her seizures.

Investigations:

EEG: Abnormal (mild)

Mild generalised irregular theta on alpha background.

This patient is a non-smoker.

2. Patient: VP

Sex: Female

Age: 35

Race: Coloured

Folder no.: 635084

History:

Onset of epilepsy: This patient has been suffering from idiopathic epilepsy for the past ten years.

Seizure pattern: Grand mal seizures.

Drug therapy: She was started on phenobarbitone (30 mg three times a day) and phenytoin (100 mg twice a day). Her seizures were never fully controlled on this drug regimen, and in 1975 carbamazepine 200 mg twice a day was introduced. There was a marked reduction in her seizure frequency and she was therefore gradually weaned off phenobarb and phenytoin, and her carbamazepine dose increased to 400 mg twice a day.

She has had no further seizures since she has been on carbamazepine monotherapy (three years). However, she suffered from frontal headaches and nausea usually around lunchtime; this corresponded to a peak in the blood level of the drug. On reduction of the morning dose from 400 mg to 300 mg her headaches have decreased in frequency and intensity. She still has good control of her seizures.

Investigations:

EEG: Normal

Alpha rhythm

This patient is a non-smoker.

3. Patient: RI

Sex: Female

Age: 19

Race: Coloured

Folder no.: 72212662

History:

Onset of epilepsy: This patient has been suffering from idiopathic epilepsy for the past six years.

Seizure pattern: Grand mal seizures.

Drug therapy: She was started on a drug regimen consisting of phenobarbitone and phenytoin. Her seizures were never fully controlled by these drugs, and therefore in 1978 sodium valproate 200 mg three times a day was introduced after discontinuing phenobarbitone. However, there was almost no improvement in her seizure frequency. It was decided, therefore, to phase out phenytoin and sodium valproate and introduce carbamazepine at 100 mg twice a day, building it up to 200 mg twice a day. On this regimen, she has had relatively good control of her seizures. However, she has suffered from severe headaches and drowsiness after taking the morning dose. This corresponded to a peak in the blood level of the drug. On reducing the morning dose from 200 mg to 100 mg the headaches have reduced in intensity with still good control of seizures.

Investigations:

EEG: Abnormal

Irregular generalised theta

No focal area of discharge

This patient is a non-smoker.

4. Patient: KS

Sex: Male

Age: 27

Race: Coloured

Folder no.: MCO 10789

History:

Onset of epilepsy: This patient sustained a head injury at the age of 12 years and developed seizures at the age of 16 years.

Seizure pattern: Grand mal seizures with focal onset.

Drug therapy: He was started on phenytoin at 100 mg three times a day. This was found to have very little effect in controlling his seizures. The regimen was therefore supplemented by carbamazepine 200 mg mane and 400 mg nocte. On this regimen he has had good control for the last five years.

He also has episodes of overt aggressive behaviour which are controlled on clozapine 200 mg nocte.

Investigations:

EEG: Abnormal

Right temporal focus consistent with temporal lobe epilepsy

N.B.: This patient is under institutionalized care.

This patient smokes 10 to 20 cigarettes per day.

5. Patient: JA

Sex: Male

Age: 38

Race: Coloured

Folder no.: MC 10146

History:

Onset of epilepsy: This patient developed idiopathic epilepsy at the age of 24 years.

Seizure pattern: This is characterised by a loss of consciousness which is preceded by gustatory and auditory auras. Post-ictally there is a marked confusional state.

Drug therapy: He was started on phenytoin 200 mg twice a day but this was found to have very little effect in controlling the seizures. Primidone was added but there was no improvement After discontinuing primidone, carbamazepine 200 mg twice a day was introduced. There was a marked reduction in seizure frequency and phenytoin was reduced to 100 mg twice a day. No seizures have been recorded for the past three years on this drug regimen.

The patient also has an underlying behavioural problem which is controlled on clozapine 200 mg nocte.

Investigations:

EEG: Normal

Alpha rhythm

N.B.: This patient is under institutionalized care.

This patient smokes 10 to 20 cigarettes per day.

6. Patient: JT

Sex: Male

Age: 21

Race: Coloured

Folder no.: MCO 1857

History:

Onset of epilepsy: This patient sustained a head injury at the age of 11 years and developed seizures at the age of 16 years.

Seizure pattern: This is characterised by episodic psychotic behaviour and depressed level of consciousness.

Drug therapy: The patient was admitted in December 1980 at Valkenberg Mental Hospital and he was diagnosed to be suffering from temporal lobe epilepsy. There was no history of previous treatment. He was therefore placed on carbamazepine 200 mg three times a day and no seizures were observed over the six months' hospital stay.

Investigations:

EEG: Abnormal

Interictal pattern with strong support for left temporal lobe focus

This patient smokes 10 to 20 cigarettes per day.

7. Patient: RT

Sex: Male

Age: 33

Race: White

Folder no.: MWC 2185

History:

Onset of epilepsy: This patient fell out of a tree onto his head at the age of 5½ years and subsequently developed seizures.

Seizure pattern: Grand mal seizures.

Drug therapy: He was on a drug regimen consisting of phenobarbitone 30 mg three times a day, phenytoin 100 mg three times a day, and carbamazepine 200 mg three times a day for a number of years. Polypharmacy did very little to control his seizures. He was gradually weaned off phenobarbitone and phenytoin and since he has been on carbamazepine monotherapy no seizures have been recorded (five years). He also has psychotic episodes which are controlled on chlorpromazine 100 mg twice a day.

Investigations:

EEG: Abnormal

Generalised irregularity

CT-Scan: Cortical atrophy

This patient smokes 10 to 20 cigarettes per day.

8. Patient: DN

Sex: Male

Age: 27

Race: White

Folder no.: MW 8416

History:

Onset of epilepsy: This patient was involved in a motor vehicle accident at the age of 5 years and sustained a fracture of the occiput, and fractures of the legs. He was unconscious for approximately 6 months. He underwent neurosurgery at the age of 13 years and subsequently developed seizures.

Seizure pattern: Grand mal seizures.

Drug therapy: He was started on phenobarbitone and phenytoin which did little to control his seizures. In 1975, carbamazepine 200 mg twice a day was introduced and he was gradually weaned off phenobarbitone and phenytoin. On this regimen he has had no attacks (three years).

He also has episodes of overt aggressive behaviour which are controlled on fluphenazine 25 mg.

Investigations:

EEG: Abnormal

Generalised irregularity

No focal onset

N.B.: This patient is under institutionalized care.

This patient is a non-smoker.

9. Patient: DH

Sex: Male

Age: 42

Race: White

Folder no.: MW 8415

History:

Onset of epilepsy: This patient has been having seizures for the past eleven years. He is also mentally retarded.

Seizure pattern: Grand mal seizures.

Drug therapy: In 1978, he was started on carbamazepine 200 mg twice a day and this was found to control his seizures effectively. There is no previous history of drug treatment.

He also has psychotic episodes which are controlled on fluphenazine 25 mg.

Investigations:

EEG: Abnormal (mild)

Mild non-specific irregularity

N.B.: This patient is under institutionalized care.

This patient is a non-smoker.

10. Patient: SS

Sex: Male

Age: 34

Race: White

Folder no.: MW 8875

History:

Onset of epilepsy: This patient was involved in a motor vehicle accident at the age of 20 years and developed seizures at the age of 24 years.

Seizure pattern: This is characterised by auditory and visual auras followed by loss of consciousness, and a post-ictal confusional state.

Drug therapy: In 1980 he was started on carbamazepine 200 mg twice a day, which resulted in good control. There is no previous history of drug treatment.

He also has episodes of psychotic behaviour which are controlled on clozapine 100 mg twice a day and chlorpromazine 100 mg nocte.

Investigations:

EEG: Normal

Alpha rhythm

This patient smokes 10 to 20 cigarettes per day.

11. Patient: BR

Sex: Female

Age: 32

Race: European

Folder no.: 53730552

History:

Onset of epilepsy: This patient has been suffering from idiopathic epilepsy since the age of 2 years.

Seizure pattern: This is characterised by nausea and vomiting which may sometimes proceed to a tonic-clonic phase.

Drug therapy: Phenobarbitone and phenytoin had very little effect in controlling her seizures. In 1972 carbamazepine in a dose of 200 mg three times a day was introduced and gradually increased to 1 200 mg daily while the other anticonvulsants were gradually discontinued. On this regimen she has had good control.

Investigations:

EEG: Abnormal

Left temporal lobe abnormality, greater anteriorly.

This patient is a non-smoker.

12. Patient: MA

Sex: Male

Age: 23

Race: White

Folder no.: MW 7003

History:

Onset of epilepsy: Seizure episodes started in 1978 at 20 years.

Seizure pattern: This is characterised by auditory and visual auras, followed by loss of consciousness.

Drug therapy: He was started on carbamazepine at a dose of 100 mg twice a day which was then increased to 200 mg twice a day. On this regimen he has had good control (one year).

He also has underlying schizophrenic behaviour and he is taking chlorpromazine 700 mg daily and orphenadrine 200 mg daily.

Investigations:

EEG: Abnormal

Right temporal focus

This patient smokes 10 to 20 cigarettes per day.

APPENDIX B

CASE STUDIES OF PATIENTS IN THE NON-RESPONDER GROUP:

1. Patient: MF

   Sex: Female

   Age: 32

   Race: White

   Folder no.: 50077494

History:

Onset of epilepsy: This patient was involved in a motor vehicle accident at the age of 17 years and subsequently developed seizures.

Seizure pattern: This is characterised by loss of concentration, nausea, biliousness (no vomiting) and abdominal distension. It may then proceed to a tonic-clonic phase and loss of consciousness.

Drug therapy: She was tried on a variety of anticonvulsants including phenobarbitone, phenytoin, sodium valproate and clonazepam with poor control.

In 1979 she was admitted for control of her seizures and started on carbamazepine 800 mg daily. On this drug regimen her attacks varied in frequency from 2 to 4 a month to several months without an attack. By the end of 1980 her attacks increased in frequency to as many as 4 in a week. Carbamazepine was increased to 1 000 mg daily but there was no improvement in seizure frequency. On this dose she suffered side-effects such as drowsiness, nausea and headaches. Early this year her dose was decreased to 800 mg

daily although the side-effects decreased slightly in intensity they were still present. She was admitted in March this year for control of her seizures. On admission, blood carbamazepine levels were 6,3 µg/ml.

Apart from her anticonvulsant drugs, the patient has also been taking domperidone (30 mg daily) and magnesium trisilicate (1 000 mg daily) for gastric complaints.

Investigations:

VDRL: Negative

CSF: Clear colourless fluid. Protein 0,1 g/l, sugar 3,2 mmol/l. No cells.

CT scan: Normal

EEG: Abnormal
Bitemporal abnormality, greater on right.
In keeping with inter-ictal record.

Course and Management:

Initially the patient was getting one attack per day at around 11 a.m. Eventually she was controlled on sodium valproate 400 mg three times a day orally and carbamazepine 200 mg three times a day orally. She was discharged on the above medication as well as sucralfate 2 tabs. twice a day orally and sennasides 3 nocte.

This patient smokes 20 cigarettes per day.

2. Patient: BG

Sex: Female

Age: 33

Race: White

Folder no.: 195328

History:

Onset of epilepsy: This patient has been having seizures since the age of 9 years.

Seizure pattern: This is characterised by an 'acidic taste' which precedes a disturbance of consciousness and amnesia for a few minutes. No clonic episodes are associated with these seizures.

Drug therapy: She was started on carbamazepine which was only partly effective in controlling the seizures. CT scan showed the presence of an epidermoid extending into the temporal horn of the lateral ventricle. In December 1977 the cyst was successfully removed surgically. She was started on phenytoin 100 mg three times a day which was then increased to 400 mg/day. Improvement in seizure frequency occurred for a while. However, in January 1980, carbamazepine was reintroduced (200 mg twice a day) after an increase in frequency of attacks. No improvement occurred. Phenytoin was withdrawn and carbamazepine increased to 800 mg daily. Still no adequate control was achieved.

Investigations:

EEG: Abnormal.

Left temporal lobe abnormality greater anteriorly on alpha background.

Course and Management:

The patient was not admitted but carbamazepine was reduced to 200 mg three times a day and sodium valproate 200 mg three times a day was introduced. On this regimen seizure frequency decreased from 8 per week to 4 per week.

This patient is a non-smoker

3. Patient: GP

Sex: Female

Age: 24

Race: Coloured

Folder no.: 9.2188294

History:

Onset of epilepsy: This 24 year old woman has been an epileptic for the past 11 years. She attributes the beginning of her problem to her being hit by her father.

Seizure pattern: Grand mal attacks with a photosensitive element.

Drug therapy: She was started on phenytoin and phenobarbitone which did little to control her seizures. Phenytoin was phased out and carbamazepine 400 mg twice a day was introduced in 1978. Since she has been on this drug regimen, her seizures improved but in the month prior to entry in the study, as many as 10 attacks were recorded. On admission, carbamazepine levels were 8,8 µg/ml and phenobarb 20 µg/ml.

Investigations:

VDRL: Negative

CSF: Clear colourless fluid, no increase in globulin, protein 0,1 g/l. No cells. Sugar 3,1 mmol/l.

CT scan: Normal

EEG: Abnormal

Inter-ictal, greater left, would query fronto-temporal focus and photosensitivity.

Course and Management:

Whilst in the ward, the patient was gradually weaned off phenobarb. Sodium valproate 200 mg three times a day

was introduced and carbamazepine was reduced to 200 mg three times a day. The patient was found to be well controlled on this drug regimen and she was discharged.

This patient is a non-smoker.

4. Patient: RA

Sex: Male

Age: 17

Race: White

Folder no.: 82332230

History:

Onset of epilepsy: This patient has a background of epilepsy since the age of 12 years.

Seizure pattern: This was initially generalised, with tonic-clonic convulsions and loss of consciousness, but towards the end of 1977 he began to see coloured dots moving up and down and obscuring his vision, followed by intense sleepiness and severe frontal throbbing headaches.

Drug therapy: The frequency of these episodes and the grand-mal seizures were reasonably well controlled by phenytoin 100 mg twice a day and carbamazepine 400 mg twice a day. After a period of time it was decided to gradually phase out phenytoin and leave the patient on carbamazepine monotherapy. The patient's seizures were still reasonably well controlled for almost a year when he had a sudden increase in his partial seizures. Increase in the dose of carbamazepine to 1 200 mg per day had no beneficial effect and he was admitted in August 1979, after a recurrence of grand mal seizures. On admission, blood level of carbamazepine was 11,2 µg/ml.

The patient was discharged on carbamazepine 400 mg twice a day and ethosuximide 500 mg twice a day. Control of the attacks although not complete appeared adequate. However, since the beginning of this year, the partial complex seizures

have increased in frequency and in the month prior to entry into the study, up to seven attacks were recorded.

Investigations:

EEG: Abnormal

Inter-ictal with right-sided focus

CT-scan: Possible temporal lobe artefact

Course and Management:

The patient complained of drowsiness, especially in the afternoon. This corresponded to a drug level of 7,1 µg/ml. However, the symptom was not severe and the neurologist thought it best to continue the same regimen for a further period.

This patient is a non-smoker.

5. Patient: MC

Sex: Male

Age: 45

Race: White

Folder no.: 57497653

History:

Onset of epilepsy: This 45 year old man has had epilepsy for the last 20 years. There is no history of head trauma.

Seizure pattern: This is characterised by an aura for 15 seconds, feeling cold and a premonition, then the seizure itself is an intense burning in the head, with very vivid distorted thoughts. The patient becomes detached and unaware of his surroundings. His pupils dilate, his eyes stare, he feels very weak. Seizures last about two minutes.

Drug therapy and progress: He was on phenobarbitone and phenytoin for 17 years which only controlled his generalised seizures. In May 1980 he was admitted because his partial complex seizures had become more frequent, as many as 12 attacks in a day. Angiography and CT-scanning showed the presence of a very large arterio-venous malformation in the right temporo-parietal region. It was decided that the lesion was too large to offer a safe chance of successful removal. The patient was therefore started on carbamazepine 400 mg mane and 500 mg nocte. His seizures have been reasonably well controlled for the last nine months but recently as many as 10 to 15 attacks have been recorded in a month.

Investigations:

EEG: Abnormal (mild)

Mild right-sided slow, greater anterior parasagittal.

Course and Management:

It was decided against admitting the patient and to continue the same drug regimen for a further period.

This patient is a non-smoker.

6. Patient: QJ

Sex: Female

Age: 17

Race: Coloured

Folder no.: 59091132

History:

Onset of epilepsy: This 17 year old girl has been an epileptic since the age of 4 years.

Seizure pattern: The fits apparently involve loss of consciousness for approximately 5 minutes with a tonic phase although there is no biting of the tongue or any incontinence. Focal abnormal movements have also been noted involving twitching of the toes, ankles and lips with some lip smacking.

Drug therapy: The patient had been medicated for years on phenobarbitone 30 mg three times a day and phenytoin 100 mg three times a day. In August 1980, the frequency of seizures increased and carbamazepine 400 mg twice a day was added. No improvement in seizure frequency occurred and approximately 20 fits per month have been recorded.

Examination: A well looking young girl with evidence of hirsutism and gum hypertrophy. There were no localising neurological signs.

Investigations:

VDRL: Negative

CSF: Clear colourless fluid, no globulin. Protein 0,1 g/l, one lymphocyte. CSF glucose normal.

CT-scan: Posterior fossa cut rotated with unexplained 1,2mm shift of the midline towards the right side.

Drug levels: On admission - phenobarb 27 µg/ml; phenytoin 30 µg/ml; carbamazepine 9 µg/ml.

EEG: Abnormal

Multi-focal epileptic inter-ictal with ? left temporal lobe focus

Course and Management:

The patient was admitted for investigation and control of her epilepsy. It was decided to stop phenobarb and phenytoin and to continue on carbamazepine alone. No seizures were recorded whilst in the ward. After being discharged, seizures have still continued even with optimal levels of carbamazepine - 8,3 µg/ml - in plasma.

This patient is a non-smoker.

7. Patient: MB

Sex: Male

Age: 19

Race: Coloured

Folder no.: 54287974

History:

Onset of epilepsy: In 1977 this patient was involved in a motor vehicle accident. On admission to hospital he underwent elevation of a depressed skull fracture and was unconscious for a number of days. He was incontinent of urine for quite a while after the injury and was unable to walk. He has had very poor vision in the left eye and seizures since the accident.

Seizure pattern: Grand mal seizures.

Drug therapy: The patient was discharged from hospital on phenytoin (300 mg daily) and phenobarbitone (90 mg daily). However, his seizures remained uncontrolled. After changing his drug regimen to phenytoin 200 mg twice a day and carbamazepine 200 mg twice a day, his seizure frequency improved for a while.

In January this year there was an increase in the grand mal attacks; carbamazepine was increased to 800 mg daily but no improvement in seizure frequency occurred. On the day of admission for investigation of his seizures, the patient had four attacks. Blood level of carbamazepine was 5 µg/ml and phenytoin 20 µg/ml.

Investigations:

EEG: Abnormal

Right hemisphere slowing, greater temporally

VDRL: Negative

CSF: Clear colourless fluid. Protein 0,1 g/l, sugar 3,2 mmol/l. No cells.

Course and Management:

Carbamazepine was increased to 300 mg three times a day and phenytoin was gradually discontinued. Whilst in the ward, no seizures were recorded (ten days). On being discharged from hospital, seizures still occurred even with blood levels of carbamazepine in the upper limit of the therapeutic range (8,2 µg/ml).

This patient smokes 10 to 20 cigarettes per day.

8. Patient: JN

Sex: Female

Age: 20

Race: White

Folder no.: 56613847

History:

Onset of epilepsy: This patient has been an epileptic since early childhood.

Seizure pattern: Grand mal seizures.

Drug therapy: Until recently her seizures have been fairly well controlled by phenobarbitone 90 mg daily and carbamazepine 1 000 mg daily. However, a month or so before entry into the study, as many as 10 to 15 seizures were recorded. Blood levels on admission were: carbamazepine 9 µg/ml and phenobarbitone 20 µg/ml.

Investigations:

VDRL: Negative

EEG: Abnormal

General spiking activity occurring intermittently and unassociated with symptoms

Course and Management:

The patient complained of nausea and headaches after the morning dose of carbamazepine. This was found to correlate with a blood level of 14,76 µg/ml. The morning dose was therefore reduced from 400 mg to 200 mg. Intravenous clonazepam was found to produce a distinct improvement in her EEG. The phenobarbitone was therefore withdrawn and clonazepam introduced. The patient was discharged on carbamazepine 800 mg daily and clonazepam 1mg daily.

9. Patient: CC

Sex: Female

Age: 16

Race: Coloured

Folder no.: 59427088

History:

Onset of epilepsy: This 16 year old girl has been an epileptic since the age of 7 years. There is no history of birth trauma or accidents, nor is there a family history of epilepsy.

Seizure pattern: Her attacks are characterised by falling to the ground after turning slowly to her right. Her upper limbs go into spasmodic fusion, as do the digits. Her lower limbs are in full extension. Her eyes turn towards the right. She then lapses into unconsciousness.

Drug therapy: She was initially treated with phenobarbitone and phenytoin; however, she developed gum hypertrophy and therefore phenytoin was phased out and carbamazepine 200 mg three times a day was introduced. Since she has been on this drug regimen, she has had a minimum of one seizure a week and was admitted for control of her epilepsy. On admission, the blood levels of carbamazepine and phenobarbitone were 7 µg/ml and 15 µg/ml, respectively.

Investigations:

VDRL: Negative

CSF: Clear colourless fluid. No increase in globulin. Sugar 3,2 mmol/l. Protein 0,2 g/l.

EEG: Abnormal

Interictal with right hemispheral focus, greater frontally.

Course and Management:

Sodium valproate 200 mg three times a day was introduced and phenobarbitone and carbamazepine gradually phased out. No fits were recorded whilst the patient was on sodium valproate monotherapy. Patient was discharged on sodium valproate 800 mg per day.

This patient is a non-smoker.

10. Patient: RC

Sex: Male

Age: 44

Race: Coloured

Folder no.: 55656854

History:

Onset of epilepsy: This patient was well until 1978 when he started having seizures. There is no history of head trauma and no family history of epilepsy.

Seizure pattern: Grand mal seizures.

Drug therapy: Phenobarbitone and phenytoin were prescribed but did not control his seizures. In 1979 phenobarbitone was withdrawn and carbamazepine 200 mg three times a day introduced. Seizures still persisted and phenytoin was increased to 500 mg daily.

In July 1980 he was admitted via the Casualty Department in status epilepticus. Blood levels of carbamazepine and phenytoin were in the therapeutic range. The patient was treated along the routine lines for status epilepticus with intravenous diazepam and phenytoin. No cause for the deterioration in seizure control could be found and the patient was discharged on phenytoin 100 mg mane, 200 mg nocte and carbamazepine 400 mg twice a day.

The patient has been complaining of increased fits recently, despite taking his medication. In June this year he was re-admitted in status epilepticus. Blood levels of carbamazepine and phenytoin were again in the therapeutic range.

Investigations:

VDRL: Negative

CT-scan: Normal

X-ray of skull: Normal

EEG: Abnormal

Bilateral generalised slow wave activity

Course and Management:

The patient responded to intravenous diazepam. The same dosage of his oral medication (i.e. 800 mg daily carbamazepine and 300 mg daily phenytoin) was continued whilst in the ward. No fits were recorded (five days) and he was discharged on the same drug regimen.

This patient smokes 20 cigarettes per day.

11. Patient: SH

Sex: Female

Age: 10

Race: White

Folder no.: 59744623

History:

Onset of epilepsy: This patient has been having seizures for the past 3 to 4 years.

Seizure pattern: These are characterised by a 'frightening feeling' after which she vomits. Occasionally she also experiences a sense of disorientation with respect to time, place and person, with no associated clonic component.

Drug therapy: She was placed on carbamazepine therapy 200 mg three times a day with initial good effect but the attacks have occurred more frequently since December 1980. She was admitted in June this year for investigation and control of her seizures.

Investigations:

CT scan: Normal

X-ray skull: Normal

VDRL: Negative

EEG: Abnormal

General slow sharp waves. Greater bilaterally, posteriorly and greater on the right. Inter-ictal pattern with strong support for right temporal focus.

Course and Management:

Patient was changed to carbamazepine 100 mg three times a day and sodium valproate 200 mg three times a day orally. No seizures were recorded whilst in the ward and she was discharged on this drug regimen.

TABLE 21: BLOOD CHEMISTRY AND HAEMATOLOGY RESULTS OF EPILEPTIC RESPONDERS

INITIALS	AGE (YRS)	SEX	WEIGHT (KG)	Na^+ mmol ℓ^{-1}	K^+ mmol ℓ^{-1}	UREA mmol ℓ^{-1}	CREATININE μmol ℓ^{-1}	PROTEIN g ℓ^{-1}	ALBUMIN g ℓ^{-1}	ALK. PHOS. UNITS	GGT UNITS ℓ^{-1}	AST UNITS ℓ^{-1}	ALT UNITS ℓ^{-1}	WCC x 10^9 ℓ^{-1}	RCC x 10^{12} ℓ^{-1}	Hb g $d\ell^{-1}$
AvA	20	F	70	141	3,9	4,4	65	66	42	48	15	12	30	4,8	4,47	13,5
VP	35	F	50	139	3,6	4,2	75	70	45	40	18	15	22	4,9	4,62	13,7
RI	19	F	52	140	4,3	5,7	82	74	49	45	2	14	21	3,9	4,19	13,5
KS	27	M	75,5	140	4,0	2,3	75	67	46	90*	61*	13	18	4,7	4,94	14,9
JA	38	M	75,2	140	3,7	3,3	70	64	39	98*	97*	27	28	9,7	4,60	14,7
JT	21	M	61,5	139	3,6	4,1	71	70	46	40	20	18	16	6,8	4,43	14,5
RT	33	M	82,5	138	8,3	2,1	70	79	44	125*	18	18	16	5,5	5,2	15,8
DH	42	M	72	141	9	4,4	74	68	44	61	19	15	16	9,3	4,9	14,4
DN	27	M	65	139	-	3,4	79	69	46	87*	45	15	18	5,7	5,4	15,8
SS	34	M	77,5	141	-	3,2	79	69	44	79	40	23	28	9,6	5,3	15,3
BR	32	F	75	140	3,6	3,1	80	65	36	21	-	18	-	6,7	3,51	11,8
MA	23	M	62	139	4,3	4,1	95	75	53	102*	20	-	-	6,5	4,44	15,2

* Results outside the normal range.

TABLE 22: BLOOD CHEMISTRY AND HAEMATOLOGY RESULTS OF EPILEPTIC NON-RESPONDERS

INITIALS	AGE (YRS)	SEX	WEIGHT (KG)	Na^+ mmol ℓ^{-1}	K^+ mmol ℓ^{-1}	UREA mmol ℓ^{-1}	CREATININE μmol ℓ^{-1}	PROTEIN g ℓ^{-1}	ALBUMIN g ℓ^{-1}	ALK. PHOS. UNITS	GGT UNITS ℓ^{-1}	AST UNITS ℓ^{-1}	ALT UNITS ℓ^{-1}	WCC x 10^9 ℓ^{-1}	RCC x 10^{12} ℓ^{-1}	Hb g $d\ell^{-1}$
MF	F	32	52	141	4,30	3,2	94	68	45	63	54*	36	58*	6,1	4,09	13,4
BG	F	33	53	142	4,10	5,7	88	65	41	62	53*	21	27	5,4	4,42	13,5
GP	F	24	46	141	3,69	3,7	80	68	47	68	-	26	-	4,6	4,32	13,4
RA	M	17	74	140	4,73	4,2	68	69	47	120*	-	29	-	4,9	4,63	14,5
MC	M	45	68	143	4,17	3,5	90	69	44	40	-	40	-	5,4	4,44	14,0
QJ	F	18	43	139	4,00	3,1	68	75	47	69	86*	19	21	4,4	4,48	13,7
MB	M	19	54	144	4,40	3,6	71	80	51	115*	-	38	-	8,0	5,15	16,5
JN	F	20	49	140	3,70	2,0	71	76	40	98*	18	15	14	9,5	4,91	13,5
CC	F	16	43	140	4,00	3,1	63	76	41	208*	8	13	21	7,6	4,16	13,8
RC	M	44	75	143	3,50	2,3	72	64	40	132*	50	20	24	18,6	5,14	14,9
SH	F	10	39	141	4,20	5,3	54	76	42	219*	-	41	-	8,8	4,81	14,4

* Results outside normal range.

APPENDIX C (contd.)

TEST	ADULT NORMAL RANGE*
SODIUM (Na^+)	135-140 mmol ℓ^{-1}
POTASSIUM (K^+)	3,5-5 mmol ℓ^{-1}
UREA	1,7-6,7 mmol ℓ^{-1}
CREATININE	75-115 µmol ℓ^{-1}
TOTAL PROTEIN	60-80 g ℓ^{-1}
ALBUMIN	35-50 g ℓ^{-1}
ALKALINE PHOSPHATASE (ALK. PHOS.)	30-85 UNITS
GAMMA-GLUTAMYL TRANSFERASE (GGT)	0-50 UNITS ℓ^{-1}
ASPARTATE TRANSAMINASE (AST)	0-40 UNITS ℓ^{-1}
ALANINE TRANSAMINASE (ALT)	0-53 UNITS ℓ^{-1}
WHITE CELL COUNT (WCC)	4-11 x 10^9 ℓ^{-1}
RED CELL COUNT (RCC)	m: 4,5-5,9 x 10^{12} ℓ^{-1}
	f: 3,7-5,3 x 10^{12} ℓ^{-1}
HAEMOGLOBIN (Hb)	m: 13,3-17,3 g dℓ^{-1}
	f: 11,6-15,6 g dℓ^{-1}

* As quoted by the Chemical Pathology and Haematology Laboratories of Groote Schuur Hospital, Cape Town.

APPENDIX D

Statistical Methods

(1) Correlation coefficient

The correlation coefficient (r) was calculated from the formula:

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2 \; \Sigma(y - \bar{y})^2}}$$

where x: is the independent variable

and y: the dependent variable.

(The relationship of y on x can be represented by a simple equation called the regression equation, i.e.: $y = a + bx$ where a = y intercept and b = slope.)

$\bar{x}$: the mean of x observations

$\bar{y}$: the mean of y observations.

In order to test whether the correlation coefficient was significant, the t value was calculated from the formula:

$$t = \frac{r\sqrt{(n - 2)}}{\sqrt{(1 - r^2)}}$$

The significance of the observed value of the correlation coefficient was then determined from the t distribution table* for - 2 degrees of freedom.

* From Siegel, S. (1956): Non-parametric statistics for the behavioral sciences.
McGraw Hill, Kogakusha Ltd.

(2) Mann-Whitney U test

The U statistic was calculated from the formula:

$$U_1 = n_1 n_2 + \frac{n_1(n_1 + 1)}{2} - R_1$$

or equivalently

$$U_2 = n_1 n_2 + \frac{n_2(n_2 + 1)}{2} - R_2$$

where

n_1 = the number of subjects in the smaller of the two independent groups

n_2 = the number of subjects in the larger group

R_1 = the sum of ranks assigned to the group whose sample size is n_1

R_2 = the sum of ranks assigned to the group whose sample size is n_2

The smaller value of U was the one used to test the null hypothesis by use of the Mann-Whitney statistical tables.*

* From Siegel, S. (1956): Non-parametric statistics for the behavioral sciences. McGraw-Hill, Kogakusha Ltd.

(3) The Randomization Test for two independent samples.

This test was used to determine whether there was a significant difference in the peak (or mean) levels of carbamazepine in patients both with or without side-effects.

Let n_1 = number of patients with side-effects

n_2 = number of patients without side-effects.

Under the null hypothesis, it was merely a matter of chance that certain levels of either drug or metabolites were labelled A and others were labelled B, where A and B were patients with and without side-effects, respectively. The assignment of the labels A and B to the levels of drug and metabolites in the particular way observed might be conceived as one of many equally likely accidents if the null hypothesis were true.

The randomization test specifies a number of extreme possible outcomes which could occur with $n_1 + n_2$ scores, and designates these as the region of rejection. When we have $\binom{n_1 + n_2}{n_1}$ equally likely occurrences under the null hypothesis, for some of these the difference ΣA (the sum of group A's scores) and ΣB (the sum of group B's scores) will be extreme. The cases for which these differences were largest constituted the region of rejection.

If α was the significance level, then the number of possible outcomes that constituted the region of rejection was $\alpha\binom{n_1 + n_2}{n_1}$. The particular outcomes chosen to constitute that number were those outcomes for which the difference between the mean of the A's and the mean of the B's was largest. These were the occurrences in which the difference between ΣA and ΣB was greatest. The null hypothesis would have been rejected at the significance level α, if the sample obtained was among those cases listed in the region of rejection.

The exact probability (one-tailed) of the occurrence of the observed levels of drug or metabolites under the null hypothesis was p = 0,95.

Therefore the null hypothesis could not be rejected.

(See Siegel, S. (1956): Non-parametric statistics for the behavioral sciences. McGraw-Hill, Kogakusha Ltd. Page 152.)

(4) The Spearman Rank Correlation Test

The Spearman rank correlation coefficient (r_s) was calculated from the formula:

$$r_s = 1 - \frac{6 \Sigma d^2}{n^3 - n}$$

where:

d^2 = the square of the difference between the ranks of the x and y variables

n = the number of observations of either x or y.

The significance of the Spearman rank correlation coefficient was determined by calculating the t value:

$$t = \frac{r_s \sqrt{(n - 2)}}{\sqrt{(1 - r_s^2)}}$$

(5) Student's t distribution

This test was used to determine whether there was a significant difference between the mean ratio of the concentrations of 10,11-dihydro-10,11-dihydroxy-carbamazepine to carbamazepine, at 6 hours after a dose, between responders and non-responders and to calculate the 95% confidence limits of the mean ratio above which we can expect a patient to be a non-responder.

The t statistic was calculated from the formula:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\left[s^2\left(\frac{1}{n} + \frac{1}{m}\right)\right]}}$$

where:

$\bar{x}$ = mean ratio for patients in the non-responder group

$\bar{y}$ = mean ratio for patients in the responder group

n = number of patients in the non-responder group

m = number of patients in the responder group

s_2 = combined variance. It was calculated from the formula:

$$s^2 = \frac{(n - 1)\, s_x^2 + (m - 1)\, s_y^2}{n + m - 2}$$

$$s_x^2 = \text{variance of } x = \left[\Sigma x - \frac{(\Sigma x)^2}{n}\right] \Big/ n - 1$$

$$s_y^2 = \text{variance of } y = \left[\Sigma y - \frac{(\Sigma y)^2}{n}\right] \Big/ m - 1$$

The 95% confidence limits above and below the mean for the non-responders were calculated from the formula:

$$\bar{x} \pm t_{0,05} \sqrt{s^2}_x$$

and similarly, the 95% confidence limit above and below the mean for the responders was calculated from the formula:

$$\bar{y} \pm t_{0,05} \sqrt{s^2}_y$$

The upper limit of the mean ratio for the responders and the lower limit of the mean ratio for the non-responders were averaged out. This gave the ratio of metabolite to drug above which a patient had a 95% chance of being a non-responder.

APPENDIX E

The Trapezoidal Rule

This method involves the description of a given plasma concentration-time curve by a function that depicts the curve as a series of straight lines, thereby enabling the area under the curve to be divided into a number of trapezoids. The area of each trapezoid is easily calculated, and the sum of all areas of all trapezoids yields an estimate of the true area under the curve.

$$\int_{t_o}^{t_n} \phi(t)dt = \sum_{i=o}^{n-1} \frac{t_{i+1} - t_i}{2} \left(C_i + C_{i+1}\right)$$

n = number of trapezoids into which the plasma curve is divided.

$\phi(t)$ = a function which describes a given plasma concentration-time curve but is linear between two plasma level-time points.

t = time

c = concentration

APPENDIX F

EQUIPMENT AND MATERIALS USED, AND SUPPLIERS

Carbamazepine 10,11-epoxy-carbamazepine 10,11-dihydro-10,11-dihydroxy-carbamazepine	Ciba-Geigy (Pty.) Ltd. 72/74 Steel Road Spartan Kempton Park Johannesburg R.S.A.
Benzene, ethyl acetate, methanol, concentrated hydrochloric and sulphuric acids (Analar, B.D.H. Chemicals, Poole, England)	Hickman and Kleber (Pty.) Ltd. P.O. Box 2953 Cape Town 8000 R.S.A.
Silica gel 60 TLC Plates, dimensions 10 x 20 cm (Merck, Darmstadt)	T & C Scientific Supplies (Pty.) Ltd. P.O. Box 2953 Cape Town 8000 R.S.A.
Universal UV lamp Type 29000 (Camag, Muttenz, Switzerland)	Labotec Cape (Pty.) Ltd. P.O. Box 773 Cape Town 8000 R.S.A.
Sample applicator for thin-layer chromatography (Eva Chrom, Switzerland) 2 µl capillary (Minicaps, EM Hirschmann, Laborglas, West Germany) Sartorius electronic micro-balance Zeiss KM3 chromatogram spectrophotometer	Zeiss West Germany Optical Instruments (Pty.) Ltd. P.O. Box 4051 Cape Town 8000 R.S.A.

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