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METABOLIC AND PHARMACOLOGICAL OBSERVATIONS  
DURING DRUG TREATMENT OF EPILEPSY

by

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A thesis submitted for the Degree of Master of Science  
in the University of Glasgow

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## SUMMARY

The thesis reports studies on patients with epilepsy. The effects of two newly introduced anticonvulsant agents are discussed with regard to the seizure control, side effects, serum levels and their effect on the metabolism of other anticonvulsant drugs when used in combination. Some of the metabolic changes induced by anticonvulsant medication are reported.

The introduction to the thesis gives an account of the general mode of action of anticonvulsant drugs, and indicates the continual search for the ideal anticonvulsant. The methods used in various analyses in this thesis are described.

The thesis is then divided into two sections. The first deals with the comparative trial of the two newly introduced anticonvulsant agents, i.e. sodium valproate (EPILIM) and clonazepam (RIVOTRIL). The two drugs were studied in a cross-over trial in 32 chronic epileptic patients who were showing poor control; one of the trial drugs was added to the existing therapy for a period of 12 weeks, after which the patients took the second drug. Sodium valproate was the more effective drug with the least side effects. However, sodium valproate had more effect on other serum anticonvulsant drug levels: it increased the serum levels of phenobarbitone and reduced those of phenytoin in some patients.

The/...

The second section of the thesis deals with two of the changes in metabolism induced by the chronic intake of anticonvulsant drugs. Chapters 3, 4 and 5 deal with the incidence of serum IgA depletion, the effect of anticonvulsants on secretory IgA, the relation of serum IgA depletion to the HLA status, and the effect anticonvulsants have on lymphocyte protein synthesis. The results show that roughly one fourth of the patients studied had reduced serum IgA levels, and this directly related to the intake of phenytoin. The reduction in serum IgA was not found to be reflected on the active form of IgA, viz. secretory IgA; although found to be reduced, this was to a much lesser degree than that of the serum. The reduction in serum IgA in patients on phenytoin was related to the higher incidence of a specific HLA pattern in these patients. HLA-A<sub>2</sub> was much more prevalent in these patients than in patients on the same medication but who did not have a low serum IgA level. Lymphocyte protein synthesis was found to be reduced in all patients on anticonvulsants when compared to controls.

During the IgA studies serum folate was measured, and it was found to be reduced in 90% of the patients. This was further studied in Chapters 7 and 8. Serum folate was found to be reduced in most of the patients studied. Those who had the lowest serum folate levels had CSF examination for folate, which was found to be reduced/...

reduced as well. The effects of treating patients with low serum and CSF folate is reported. The treatment was with folic acid or with 5-formyltetrahydrofolate. The effects of treatment on seizure frequency, serum anticonvulsant levels and CSF amine metabolites (HVA and HIAA) were studied. The results show that CSF folate increased more with 5-formyltetrahydrofolate treatment. Serum phenytoin levels were reduced with treatment, and the levels of HVA and HIAA were reduced with 5-formyltetrahydrofolate; there was, however, no effect on seizure frequency.

## UNITS USED

Kg	kilogram $1 \times 10^3$ gram
mg	milligram $1 \times 10^{-3}$ gram
ug	microgram $1 \times 10^{-6}$ gram
ml	millilitre, $1 \times 10^{-3}$ litre
dl	decilitre $1 \times 10^{-1}$ litre
$\mu$ l	microlitre $1 \times 10^{-6}$ litre
$\mu$ mol/l	micromoles per litre $1 \times 10^{-6}$ moles per litre
nmol/l	nanomoles per litre $1 \times 10^{-9}$ moles per litre
min	minute
$^{\circ}$ C	degree centigrade

## ABBREVIATIONS

A.I.P.	Automated immunoprecipitation
CSF	Cerebrospinal fluid
DNA	Desoxy-ribonucleic acid
EEG	Electroencephalography
GABA	Gamma aminobutyric acid
HIAA	5-hydroxy- $\beta$ -indole acetic acid
HLA	Histocompatibility lymphocyte antigen
HVA	Homovanillic acid
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
M.N.A.	Mean normal adult
phenytoin	di-sodium phenylhydantoin
R.I.D.	Radial immunodiffusion
RNA	Ribonucleic acid
t.i.d.	ter in diem
SD	Standard deviation

## INTRODUCTION

Epilepsy affects one in 200 of the population (Brain and Walton, 1969). In two-thirds of all patients, it takes a chronic form and consequently patients continue taking anticonvulsants. This makes anticonvulsant drugs one of the most commonly taken drugs. Because of the large number of anticonvulsant drugs which are only effective in certain types of epilepsy, a large percentage of medical practitioners still have difficulties in choosing the right drug and dosage for their patients. In this thesis, the effects of new drugs introduced into the armamentarium of anticonvulsant agents, and some of the effects on metabolism produced by anticonvulsants are studied.

Modern concepts about epilepsy were started by the eminent neurologist Hughling Jackson in the 19th century. He postulated that epilepsy is an intermittent disorder of the nervous system due presumably to a sudden, excessive, disorderly discharge of cerebral neurons.

The problem of treating seizures is still far from ideal. This is mainly because we are still not treating the original defect causing the seizures, but mainly trying to stop the propagation of the 'disorderly discharge'. Anticonvulsant drug therapy is only one pillar in the management of the epileptic patient.

Any/...



Any general depressant of the nervous system will diminish or stop epileptic activity. Anticonvulsants act mainly by preventing the spread of the discharge from an epileptic focus. There are many possible ways in which anticonvulsants might operate to abolish or attenuate seizures:

1. Effects on non-neural lesions, such as normalisation of the ischaemic blood supply of an epileptic focus; however, it is notable that autonomic blocking or facilitating agents have little or no value in the treatment of epilepsy.

2. Effects confined to the pathologically altered neurons of the seizure focus, to prevent their excessive discharge; such an action is suspected, but not proved in the ability of increased blood levels of CO<sub>2</sub> to abolish clinical petit mal seizures.

3. Effects on normal neurons to prevent the detonation by the seizure focus. This probably includes most if not all the presently available anticonvulsant drugs; all can be demonstrated to modify the ability of the brain to respond to various seizure evoking stimuli. The particular physiological effects might include reduction of post-tetanic potentiation, elevation of excitatory synaptic threshold, potentiation of either pre-synaptic or post-synaptic inhibition, and prolongation of/...

of the refractory period.

### The ideal anticonvulsant

The ideal anti-epileptic agent should be capable of suppressing seizures at a dosage that does not lead to sedation or other central toxicity, it should be well-tolerated by the oral route, should not lead to tolerance or withdrawal symptoms, should be cheap, long-acting, devoid of systemic toxic effects and free from even occasional idiosyncratic effects. The drug should preferably have a wide margin of safety, with a wide range of activity to control all types of clinical epilepsy through exerting its effects directly on the seizure focus (Toman, 1970). No such drug exists or is likely to be discovered for some time.

Various anticonvulsant drugs have been introduced mainly since the beginning of this century. After the use of Bromide since 1857, the first of the modern anticonvulsant agents was introduced, i.e. Barbiturate derivatives. In 1903 Barbitone and in 1912 phenobarbitone were introduced. In 1938 after an intensive search, Diphenylhydantoin (phenytoin) was introduced by Merrit and Putnam. Since then, several other anticonvulsants have been introduced. However, the two most commonly used anticonvulsant agents today are still phenytoin and phenobarbitone/...

phenobarbitone.

To measure the therapeutic and the side effects of an anticonvulsant involves reliable seizure recording, good compliance (which is best monitored by serum levels), avoidance of contributory factors, knowledge of the pharmacokinetics of the drug, and lastly giving enough time for observation. In the work reported in this thesis, these criteria were to a very large extent adhered to.

This thesis is divided into two sections: the first deals with the effects of two anticonvulsant drugs, namely sodium valproate and clonazepam. Sodium valproate (Di-propylacetate; EPILIM, Reckitt and Colman) is a newly introduced drug in this country, and a lot of its properties are still not worked up. The drug is derived from a fatty acid with a different mode of action when compared to other anticonvulsants in use. The postulated mode of action of sodium valproate is through increasing the levels of gamma-amino butyric acid (GABA) by inhibiting GABA transaminase. The brain concentration of GABA has been shown to increase following the administration of certain anticonvulsants, and its concentration has been shown to be reduced following certain convulsants (Meldrum, 1975). Sodium valproate has been shown to increase the levels of GABA in both the cerebral cortex/...

cortex and the cerebellum, with complete protection against audiogenic seizures (Ciesielski, Maitre, Cash and Mandel, 1975). The effectiveness of sodium valproate, when added to other drugs, has been shown by previous workers (Jeavons and Clark, 1974; Richens and Ahmed, 1975).

The other drug which is reported in this thesis is clonazepam (RIVOTRIL, Roche Products). It is a benzodiazepine and its effectiveness and side effects are still being assessed (Nanda, Keogh, Lambie, Johnson, Melville and Morrice, 1976). It was felt that a comparative trial of the effects of both drugs on seizure frequency, anticonvulsant serum levels and toxicity was needed. This will be reported upon in Section 1 of this thesis.

Patients who take drugs for life present a unique opportunity to study various aspects of metabolism, which may be changed due to direct or indirect effect of the drug intake. Anticonvulsants affect the immune system, particularly Immunoglobulin A (IgA); this has been shown to be reduced, as well as lymphocyte protein synthesis (Sorrell and Forbes, 1975). Another immunoglobulin, namely IgG, was reported to be involved, and the serum IgA depletion was not correlated with phenytoin therapy (Slavin, Fenton, Laundry and Reynolds, 1974). IgA function is that of protecting the mucous membranes and  
it/...

it is secreted by plasma cells in the submucous layer of the mucous membranes. IgA is found in nearly all body secretions (Walker and Isselbacher, 1977). The secretory IgA is the functional form, and it has been reported to be reduced in epileptic patients on anti-convulsants (Aarli and Tønder, 1975). However, this may not be the case because serum and secretory IgA levels do not necessarily follow the same pattern (Tomasi, 1968). The cause of the serum IgA deficiency is not known, but there is a suggestion that it could be genetic (Fontana, Grob, Sauter and Joller, 1976). The serum and salivary IgA status will be reported upon in Chapters 3, 4 and 5 of this thesis in relation to anticonvulsant intake and drug levels, folate status, and HLA typing. In addition, lymphocyte protein synthesis as a marker of T-cell function is also studied.

The other aspect of metabolism studied in this thesis is that of folate. It is well known that anticonvulsants produce changes in the folate metabolism. This was reported as increased incidence of macrocytosis (Hawkins and Meynell, 1958). Several other workers since have assessed the effect of anticonvulsant drugs on folic acid metabolism. Cerebrospinal fluid (CSF) folate is normally higher than that of the serum (Wells and Casey, 1967). This was reported to be reduced also in epileptic patients/...

patients on anticonvulsants (Reynolds, Mattson and Gallagher, 1972). The CSF levels of folate were reported only to increase when the reduced form of folic acid (5-formyltetrahydrofolate) was used for treatment, and not with folic acid itself, indicating perhaps that anticonvulsants interfere with the conversion of folic acid into the active form, i.e. 5-methyltetrahydrofolate (Mattson, Gallagher, Reynolds and Glass, 1973). The effects of treating folate deficient epileptic patients has been controversial as regards their mental state and seizure frequency. Worsening of seizure frequency has been reported (Reynolds and Wales, 1967). However, this was not confirmed by other workers (Jensen and Olesen, 1970). The amine metabolites in the CSF, namely 5-hydroxy-3-indole acetic acid (HIAA) and homovanillic acid (HVA) have been reported to be increased in epileptic patients (Chadwick, Jenner and Reynolds, 1975). The relation of this finding to folate metabolism is uncertain, and it is controversial whether folate acts as a methyl donor in amine metabolism (Reynolds, Chadwick, Jenner and Chanarin, 1975). Administration of folic acid to epileptic patients did not significantly lower the CSF HVA or HIAA (Hunter, Barnes, Curzon, Kantamaneni and Duncan, 1971).

The effects of treatment of folate deficient epileptic patients with either folic acid or 5-formyltetra/...

tetrahydrofolate in relation to CSF folate, and with particular reference to CSF amine metabolites, is reported in Chapters 6 and 7.

## METHODS

## PATIENTS

The studies reported in this thesis were done on patients suffering from various types of epilepsy. They were mainly patients from the Epilepsy Centre, Quarrier's Homes, Bridge of Weir, Renfrewshire, and some patients attending the Institute of Neurological Sciences, Southern General Hospital, Glasgow. The number of patients and the particular type of epilepsy will be given in each chapter as they were included in various studies.

## TECHNIQUES

a) Blood sampling

Blood samples were taken by venepuncture from an antecubital vein after cleaning the area with spirit. If serum anticonvulsants were to be measured, the patients would be fasted overnight, but in the case of anticonvulsants with a short half life, i.e. primidone and sodium valproate, the blood samples were taken two hours after the morning dose. Blood was collected in plain plastic tubes. The serum was separated and stored at  $-20^{\circ}\text{C}$  until the time of assay.

b/...



b) Cerebrospinal fluid sampling

Cerebrospinal fluid (CSF) samples were taken in the morning in fasting patients. The patients were kept lying in bed after awakening in the morning and lumbar puncture was performed using gauge 20 lumbar puncture needles while the patients were in the left lateral position. The skin was cleaned with Hibitane and the disc space identified. Local anaesthesia in the form of Lignocaine 2% was infiltrated before inserting the spinal needle. Around 8 mls of CSF was collected in two separate containers. The first 5 mls were used for HVA and HIAA assay, and the rest for CSF folate. The samples were stored at  $-20^{\circ}\text{C}$  until they were analysed.

c) Parotid saliva sampling

Sampling from one of the parotid duct openings was done in the morning after an overnight fast, and following a mouth-rinse with water. The saliva was not stimulated. It was collected using a modified Carlson-Crittenden collecting cup (Schaeffer, Sproles and Krakowski, 1973). This cup consists of two rounded compartments, one inside the other, each connected to the outside by a separate tubing. The inner one is applied over the parotid duct opening and the outer one is connected to continuous suction. Saliva is collected through the tubing that comes from the inner ring (Fig. i). Around 1 ml of unstimulated parotid saliva was collected over 30/...

Fig. i Carlson-Crittenden cup, for collecting parotid saliva. The saliva accumulates in the inner compartment, and continuous suction is applied to the outer compartment.



30 - 45 min and stored at  $-20^{\circ}\text{C}$  until the time of analysis.

The biological fluids were examined by a series of techniques for analyses of anticonvulsants, immunoglobulins, folates, catecholamine and serotonin metabolites. I was helped in some of the techniques as part of this work was a collaboration with certain colleagues, to whom I am grateful. Details of the assays are given below.

### ASSAYS

#### a) Anticonvulsant drug assays

Phenytoin, phenobarbitone and primidone were measured by gas-liquid chromatography using a Pye Unicam gas chromatograph (Type 104) equipped with dual flame ionization detectors. Separation was achieved by temperature programming on a 3 ft. (91.44 cm) glass column of OVI (80-100 mesh), Pye Unicam Ltd. The method used is a variation of that described by Goudie and Burnett (1973) using p-tolyl phenylhydantoin as internal standard and on column methylation with trimethylphenyl ammonium hydroxide.

Sodium valproate was estimated by an isothermal gas liquid chromatographic method using a 3 ft. (91.44 cm) column of 2% WG/11 on Chromosorb W (HP) 80-100 mesh. The method is as follows:- pipette 100  $\mu\text{l}$  serum, 50  $\mu\text{l}$  internal standard/...

standard solution (200 mg% cyclohexane carboxylic acid in 1 N H<sub>2</sub>SO<sub>4</sub>), and 100 µl Dichloromethane into a 5 ml conical tube; vortex 30 seconds, centrifuge. If organic layer remains cloudy stir with fine wire and re-centrifuge. Draw about 1 µl air into 5 µl syringe and place needle in organic layer (no need to remove upper layer), eject air and draw in 2-3 µl lower layer for GLC. The oven temperature was 150°C and the detector block temperature was 250°C. The carrier gas used was oxygen-free nitrogen (B.O.C. Ltd.) at a flow rate of 30 ml/min.

Clonazepam was measured by radio-immunoassay (O'Kelly, 1975) at the Psycho-endocrine Research Centre, St. James Hospital, Dublin.

The techniques using the gas chromatograph were all in operation in the laboratory when I joined it. I became proficient in the techniques and many of the results therefore depend upon assays carried out by me.

b) Immunoglobulin estimation

Serum and salivary immunoglobulin levels (IgG, IgA, IgM) were measured by radial immunodiffusion and by the automated immunoprecipitation (A.I.P.) system.

i) Radial immunodiffusion

This technique utilises a precipitin reaction in which/...

which the internal reactant (specific antibody) is incorporated in a buffered agar medium. Serum containing the immunoglobulins (Antigen) is placed in a well centred in the immunodiffusion slide. By providing an unlimited amount of the antibody in the gel there will be no undue restriction on the diffusion of the antigen, resulting in a precipitin zone directly related to the amount of the antigen present. Reference sera were used with a known amount of antigens and their results plotted. The concentration of the unknown sera can be determined by reference to this graph (Fahey and McKelvey, 1965). Commercial radial immunodiffusion plates were purchased from ICL Scientific. Many of the immunoglobulin assays were done by myself in this method, but they were repeated using nephelometry as explained below.

ii) Nephelometry

The Technicon A.I.P. system was used to measure serum and salivary immunoglobulins, utilising Technicon reference sera. The nephelometer measures the light scattered from the sample, which depends more on the number of molecules (Antigen - Antibody complexes) than on the size of the molecule. This may be important when measuring the bigger size salivary IgA, bearing in mind that serum IgA standards were/...

were used as a reference for both serum and salivary IgA.

The method for the latter assay was carried out by the Department of Haematology, Southern General Hospital, Glasgow. I am grateful for this collaboration.

c) Serum and CSF folate

A modification of the Lactobacillus Casei method (Herbert, 1966) was used, with a dilution of 1:20 for the serum, 1:100-1:200 for the CSF folate. The assay was carried out in the Haematology Department of the Southern General Hospital. I am grateful for this collaboration.

d) Homovanillic acid and 5-hydroxy-3-indole acetic acid in CSF

Samples of CSF were analysed with a spectrophotofluorimeter (Aminco Bowman 125, fitted with an off-axis ellipsoidal condensing mirror), by the method of Ashcroft, Crawford, Dow and Goldberg (1968). Each batch of samples was analysed together with a set of 'standards' prepared in triplicate containing known quantities of HVA and HIAA in 5 ml of double distilled water. A 'blank' of 5 ml double distilled water was also used. This assay was carried out in collaboration with Dr. H. Keogh to whom I am grateful.

## SECTION I

As mentioned in the introduction, the control of seizures in epilepsy by use of anticonvulsants is still far from ideal, and some patients still have a large number of seizures in spite of being on multiple anticonvulsant drugs and these patients often present a social as well as a medical problem because of their poor seizure control and the deranged mental state that often accompanies that.

Patients studied in this section fall into the category mentioned above and they are all chronic epileptics who are residential in an Epileptic Centre; despite multiple drug therapy and supervised intake of drugs, they still have a large number of seizures, which makes them unable to cope normally in the society.

It was felt that the group of patients mentioned above would be very suitable to study the effects of two recently introduced anticonvulsant agents, i.e. Sodium Valproate (EPILIM, Reckitt & Colman) and Clonazepam (RIVOTRIL, Roche Products), because of the poor seizure control and the effect that these drugs may have on other anticonvulsants. The effect of these two drugs and their side effects will be discussed in this section.



## CHAPTER 1

Comparison of sodium valproate (Epilim) and clonazepam (Rivotril) in intractable epilepsy.

## INTRODUCTION

The introduction of new anticonvulsant drugs needs their assessment both individually and comparatively. Although two recently introduced drugs, clonazepam (Rivotril, Roche Products Ltd.) and sodium valproate (Epilim, Reckitt & Colman), have now been subjected to several different studies independently, comparative assessment of their value is still needed.

Clonazepam is a benzodiazepine closely related to other members of the group such as nitrazepam, diazepam and chlordiazepoxide (Fig. 1.1). It was shown to suppress generalised EEG abnormalities more readily than focal abnormalities (Gastaut, Courjon and Poire, 1971; Rossi, Di Rocco and Maira, 1973). It also enhances polysynaptic inhibitory processes at all levels of the central nervous system (Young, Zukin and Snyder, 1974).

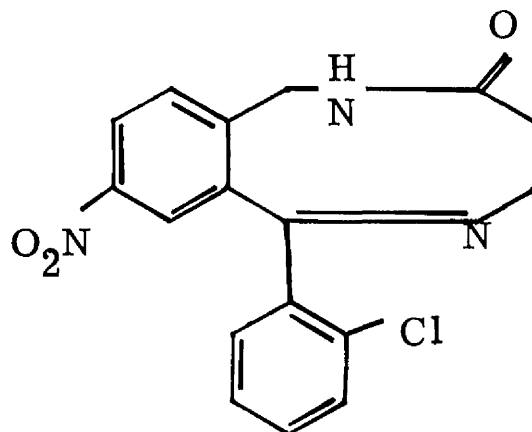
Sodium valproate is structurally unrelated to any of the known groups of anticonvulsant agents being a sodium salt of a fatty acid (Fig. 1.2) and its mode of action is postulated to be through interference with the GABA shunt of the tricarboxylic acid cycle (Fig. 1.3). The maximum seizure protection afforded by sodium valproate/...

Fig. 1.1 Structure of clonazepam (RIVOTRIL, Roche Products).

Fig. 1.2 Structure of sodium valproate (EPILIM, Reckitt & Colman).

CLONAZEPAM (Rivotril) Roche Products Ltd.

a BENZODIAZEPINE compound



SODIUM VALPROATE (EPILIM, Reckitt & Colman)

Salt of a fatty acid.

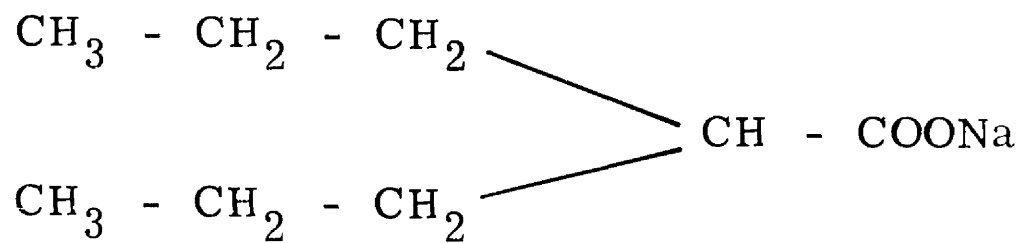
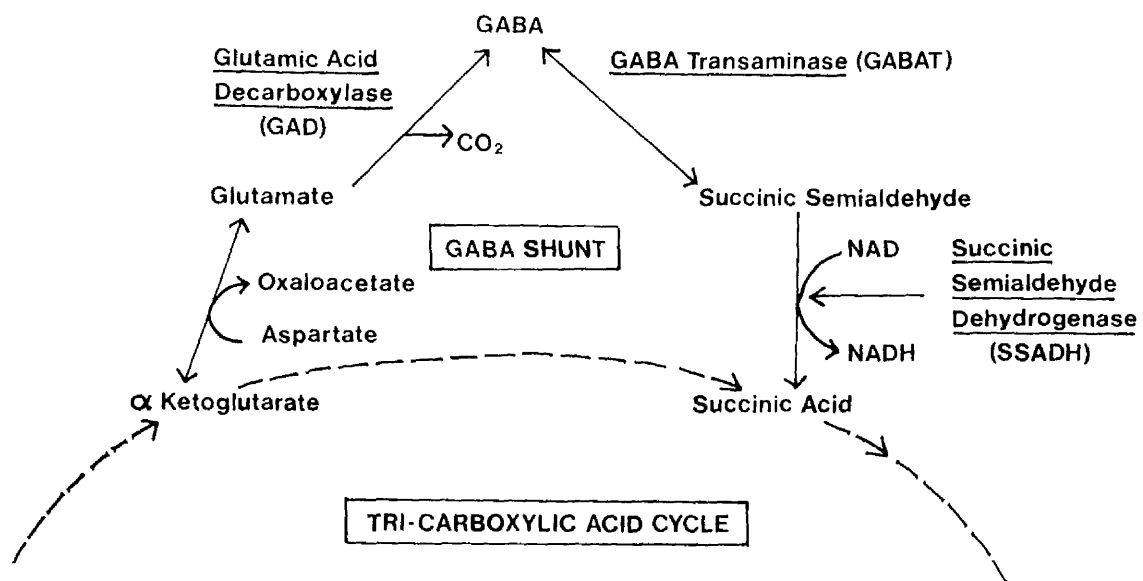


Fig. 1.3 Tricarboxylic acid cycle (courtesy of Dr. P.K.P. Harvey, Department of Biochemistry, Imperial College, London), showing the possible site of action of sodium valproate by inhibiting GABA transaminase and increasing GABA levels.



valproate coincided with the maximum elevation of total brain GABA concentrations, but to produce this a very high dose of sodium valproate was needed, 400 mg/kg in mice (Simler, Ciesielski, Maitre, Randrianarisoa and Mandel, 1973).

One comparative trial between sodium valproate and clonazepam has been reported (Lance and Anthony, 1977), but it was carried out as an open trial, sodium valproate being given to patients in whom other drugs, including clonazepam, had not produced adequate control. The drugs were not therefore directly compared in the same patients. A cross-over trial was carried out in a group of patients who were poorly controlled on existing anticonvulsant therapy. Serum levels of sodium valproate and clonazepam as well as phenytoin, phenobarbitone and primidone were measured to try to see the correlation between the dose, serum level and seizure control, as well as the effect of the new drugs on the serum levels of other anticonvulsants, as will be reported in the next chapter.

## METHODS

### Patients

Thirty-two adult chronic epileptic patients, all residents in the Epilepsy Centre, Quarrier's Homes, Bridge of Weir, Renfrewshire, were studied. They were 15 males and 17 females, aged 21-63 years (mean 36 years). All of the patients had epilepsy for ten or more years and/...

and none had evidence of a progressive disease. They were all already on anticonvulsant medication: phenytoin (20 patients), phenobarbitone (17), primidone (14), carbamazepine (10), ethosuximide (2) and pheneturide (1).

The criteria of selection were that they had had more than five seizures per month for the preceding three months and in two separate estimations of anticonvulsant drug concentrations in that period, the values were within the accepted therapeutic ranges for phenytoin (40-80  $\mu\text{mol/l}$ ) or phenobarbitone (40-160  $\mu\text{mol/l}$ ).

Twenty-nine patients had frequent major seizures (generalised tonic-clonic fits), in addition to frequent minor seizures, absences, myoclonic attacks, focal attacks, including adersive attacks, Jacksonian attacks and temporal lobe attacks. These patients showed a variety of EEG abnormalities. Thirteen had bilateral slow wave or spike wave activities with no definite localisation; nine had temporal lobe foci; five had other focal lesions and two showed such low voltage activity that focal lesions could not be identified. The remaining three patients had minor seizures only and the EEGs showed respectively centrencephalic, temporal lobe and focal fronto-parietal abnormalities.

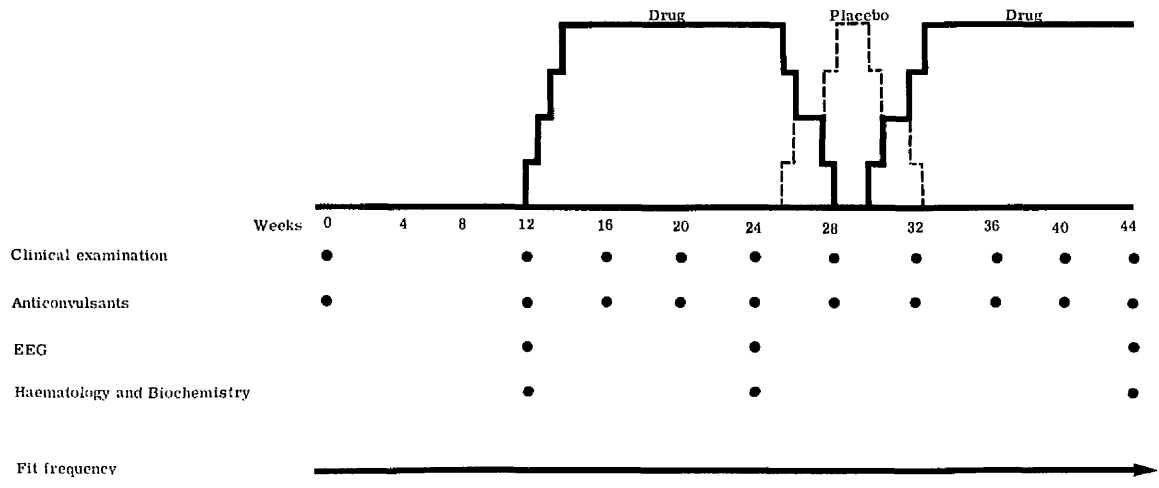
Trial Design/...

Trial Design (Fig. 1.4)

The trial consisted of three consecutive periods, each of twelve weeks. Patients who satisfied the criteria for selection given above were observed for twelve weeks during which serum concentrations of their anticonvulsants were estimated twice. The patients were then divided into two groups each of 16 patients. One was given sodium valproate (400 mg four times daily, the dose being increased in four steps over a two-week introduction period), and the other was given clonazepam (2 mg three times daily, this value being achieved over two weeks in three steps). The trial drug was added to the patients' earlier regime. As far as possible, serum concentration of phenytoin or phenobarbitone was kept constant in all patients by reducing their dosage if they showed an increase of more than 15%. After 12 weeks on the first drug, each group crossed over to the other drug. The change was made over a six week period using a placebo matching the first trial drug. During the first two weeks of the cross-over period the first drug was replaced by placebo; in the second two weeks the patient continued on placebo only and in the third two weeks the second drug was built up and the patient then continued on the second drug for a further twelve weeks. There was therefore a period of two weeks during which the patients were on placebo only, to reduce the possibility/...



Fig. 1.4 Trial design; showing the cross-over fashion after 12 weeks on either drug. The frequency of each observation is indicated by (.), as it was done in the study. (-) indicates one of the trial drugs. (---) indicates that the patients received placebo matching the drug used in the first 12 week period.



possibility of interactions between the two trial drugs.

Seizures were recorded by nursing staff as major and minor seizures as explained above. The reduction in seizure frequency is graded into 100% reduction, 75-100% reduction, 50-75% reduction, 50% reduction and worse (more seizures during drug treatment). The significances of difference were assessed by the Wilcoxon signed-ranks test.

#### Anticonvulsant analysis

Sodium valproate was measured by Gas Chromatography. Serum clonazepam levels were measured by radio-immunoassay (see under METHODS).

### RESULTS

#### Seizure frequency

Of the 32 patients who started the trial, 15 patients completed all parts of the trial. The remaining 17 patients completed one part of the drug trial only, 12 completing the sodium valproate period and five completing the trial with clonazepam. Eight of these 17 patients left the trial after the first drug period for various reasons unrelated to the drug therapy. Another nine patients stopped taking the trial drug because of side effects; eight were on clonazepam and one on sodium valproate/...

valproate.

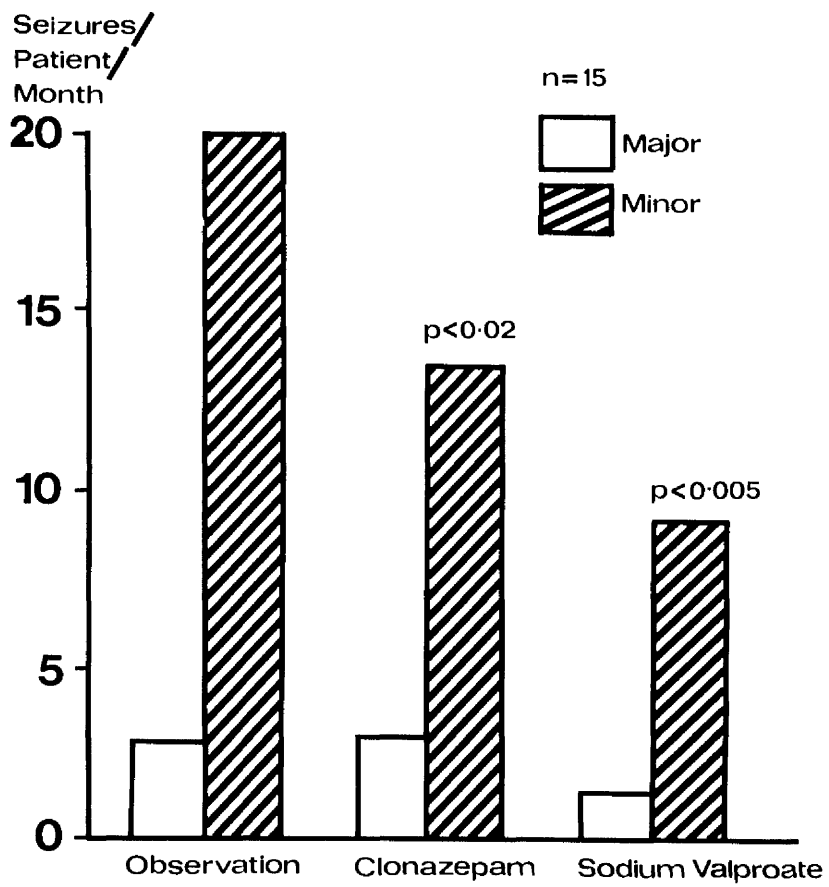
When the average number of seizures per patient per month before and after treatment were compared in those 15 patients who completed the trial, a significant reduction in the frequency of minor seizures was found both with sodium valproate ( $P < 0.005$ ) and clonazepam treatment ( $P < 0.02$ ). Major seizures were also reduced with sodium valproate, but this failed to reach significance (Fig. 1.5). Sodium valproate had the greater effect and reduced seizure frequency overall by more than 50%. The changes in seizure frequency, according to the type of epilepsy, are given in Tables 1.1 and 1.2.

#### Side effects

The main side effects with sodium valproate were drowsiness (25% of patients) and ataxia (17% of patients). These were abolished in all but one patient by reducing the serum concentration of phenobarbitone or primidone. In one patient the drug was stopped because of these side effects. Gastro-intestinal symptoms (nausea, vomiting, diarrhoea) were noted in three patients during sodium valproate treatment, but it was mild and transitory. With clonazepam 65% of patients showed drowsiness and 55% of patients were ataxic. Because of side effects, the dose of clonazepam was reduced in 13 of the 29 treated patients from 6 mg to 1-5 mg/day and eventually stopped in eight patients.

#### Serum anticonvulsant concentrations/...

Fig. 1.5 Histogram showing the average number of seizures/patient/month in the 15 patients who completed the trial, during observation, sodium valproate, and clonazepam therapy. Differences were assessed by the Wilcoxon signed-ranks test. (□) major seizures, (▣) minor seizures.



MAJOR SEIZURES

TYPE OF EPILEPSY	SODIUM VALPROATE				CLONAZEPAM					
	No.	Reduction in seizures			No.	Reduction in seizures				
		100%	75-100%	50-75% Worse		100%	75-100%	50-75% Worse		
GENERALISED	12	2	2	3	8	-	-	1	5	2
FOCAL										
Temporal Lobe	7	3	-	2	7	-	1	1	1	4
Focal Motor	3	-	2	-	2	-	-	-	-	2
UNCLASSIFIED	2	1	-	1	1	1	-	-	-	-
TOTAL	24	6	4	5	18	1	1	2	6	8

Table 1.1: The effect of treatment with clonazepam and sodium valproate on the frequency of major seizures in all the patients who were treated with one or both drugs (100% = no seizures).

MINOR SEIZURES

TYPE OF EPILEPSY	SODIUM VALPROATE				CLONAZEPAM							
	No.	Reduction in seizures			No.	Reduction in seizures						
		100%	75-100%	50-75%		50% Worse	100%	75-100%	50-75%			
GENERALISED	13	1	-	7	4	1	9	-	-	3	4	2
FOCAL												
Temporal Lobe	8	-	1	1	5	1	7	-	-	-	3	4
Focal Motor	4	-	1	1	1	1	3	-	1	-	1	1
UNCLASSIFIED	2	1	-	-	1	-	1	-	1	-	-	-
TOTAL	27	2	2	9	11	3	20	-	2	3	8	7

Table 1.2: The effect of treatment with clonazepam and sodium valproate on the frequency of minor seizures in all the patients who were treated with one or both drugs (100% = no seizures).



Serum anticonvulsant concentrations

The serum concentrations of sodium valproate for all the patients treated with the drug, all receiving a dose of 1600 mg daily, ranged from 224-882  $\mu\text{mol/l}$  (mean  $497 \pm 184$  SD  $\mu\text{mol/l}$ ). The variation was generally fairly small, with 21 of the 26 (blood sample was not obtained from one patient) showing concentrations within the range of 350-700  $\mu\text{mol/l}$ . There was no apparent relationship between the serum concentrations and the therapeutic effect: 18 patients who showed a significant (>50%) reduction in the total number of seizures had a mean concentration of 490  $\mu\text{mol/l}$  and eight patients who showed little or no improvement had a mean concentration of 513  $\mu\text{mol/l}$ .

The serum concentrations of clonazepam showed rather more variation. The mean serum concentration for 23 patients in whom blood samples were obtained while they were receiving 6 mg of clonazepam daily was  $82 \pm 38$  SD nmol/l, but a number of patients showed lower serum concentrations throughout the major part of the trial since doses were later reduced. Twelve patients on doses ranging from 1 to 5 mg daily (mean 2.9 mg) showed a mean serum concentration of  $29 \pm 19$  SD nmol/l. A possible correlation between serum concentrations and therapeutic effect was seen in the fact that (considering the 20 patients who completed the clonazepam treatment period) the/...

the four patients who showed a significant ( $> 50\%$ ) reduction in total seizures showed a serum clonazepam concentration greater than 50 nmol/l whereas eight of the 16 patients showing no improvement had levels below 50 nmol/l.

In order to correlate the serum levels of clonazepam and sodium valproate to the patients' anticonvulsant medication dosage, a system of drug units was used (Richens and Rowe, 1970). In this system, one unit represents each 50 mg of phenytoin or 30 mg of phenobarbitone, and 1.5 units represents 250 mg of primidone. Fig. 1.6 shows the relation of clonazepam concentrations to the drug units. There is a negative correlation between the two (patients who were on more drugs had lower serum clonazepam concentrations), while there was no correlation with sodium valproate (Figs. 1.6 and 1.7 respectively).

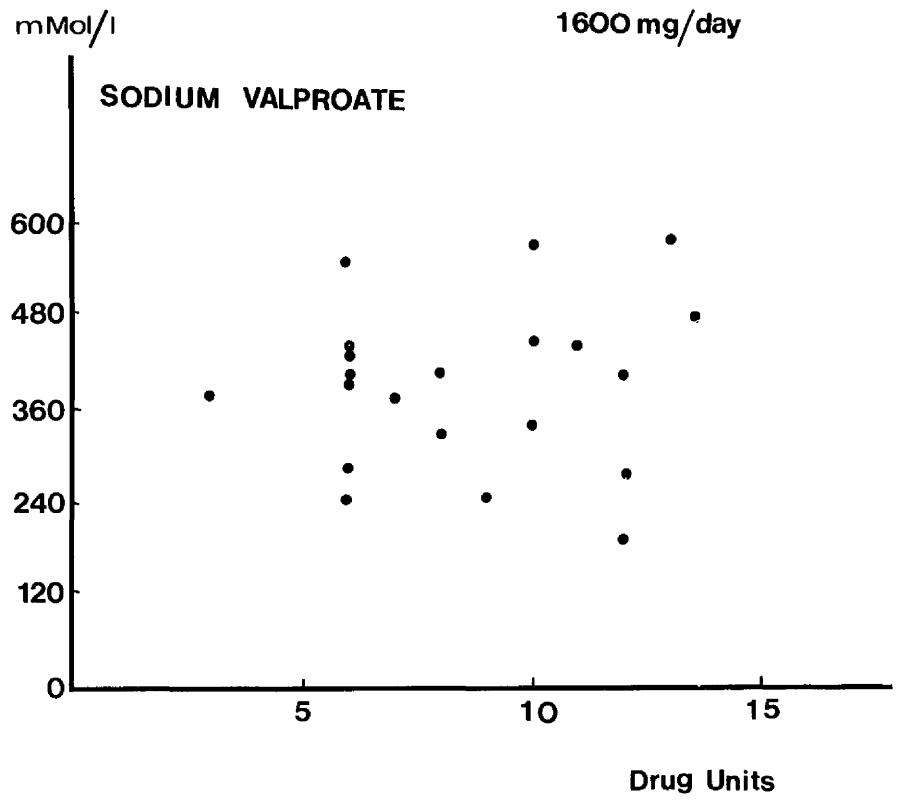
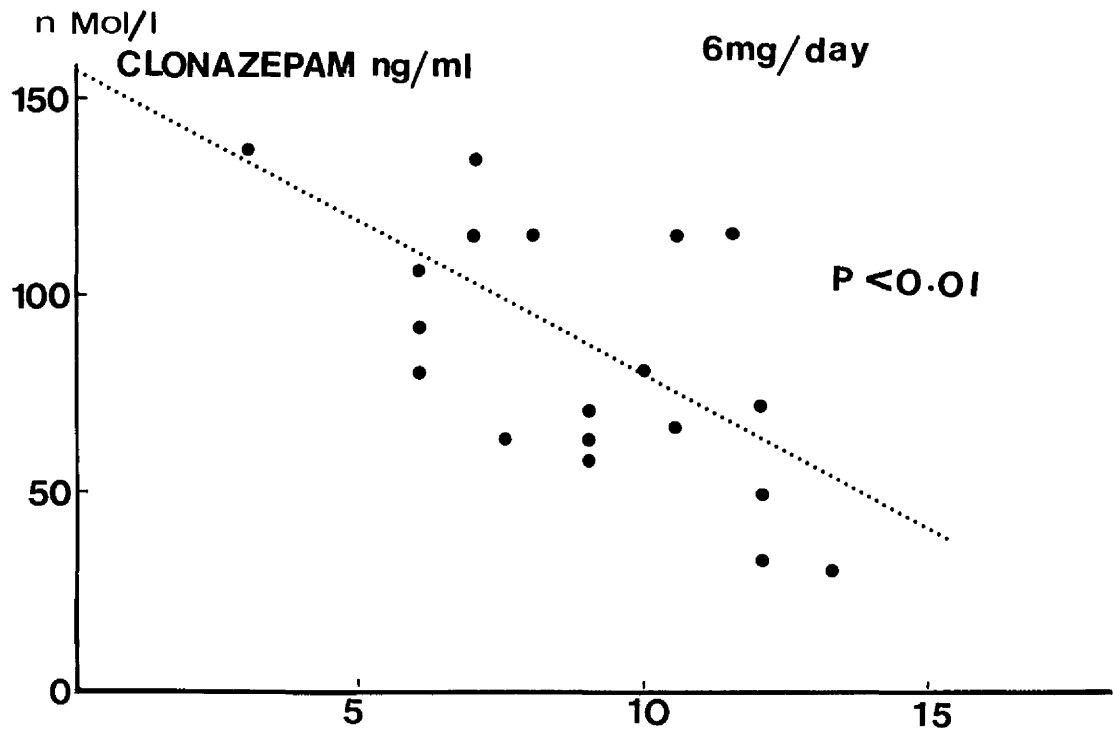
The effects of both drugs on other anticonvulsant serum concentrations (phenytoin, phenobarbitone and primidone), as well as the effects these latter mentioned drugs have had on the trial drugs, will be discussed in the following chapter.

## DISCUSSION

Both sodium valproate and clonazepam reduced the frequency of minor seizures when added to the existing therapy/...

Fig. 1.6 The correlation of serum clonazepam levels in nmol/l in 19 patients, all taking a dose of 6 mg/day, to the drug units (amount of other anticonvulsants the patients were taking). Negative correlation ( $P < 0.01$ ).

Fig. 1.7 Correlation between the serum sodium valproate level in  $\mu\text{mol/l}$  in 21 patients, on a dose of 1600 mg/day, and the drug units (amount of other anticonvulsants the patients were taking). No significant correlation.



therapy of patients whose fits had proved difficult to control. Clonazepam was generally less effective than sodium valproate although it appeared to be of value in a few patients. Major seizures were also reduced overall by sodium valproate, but this did not reach a level of significance. Sodium valproate appeared to be more effective in reducing seizures in patients with generalised epilepsy than in those with focal epilepsy and this accords with findings in other centres (Jeavons and Clark, 1974; Meinardi, 1971).

Drowsiness was a major problem with clonazepam, and this required reducing the dose or stopping the drug in a considerable number of patients. Drowsiness also occurred in patients during sodium valproate treatment, but appeared to be due to elevation of serum phenobarbitone concentrations and was abolished by reducing the dose of phenobarbitone or primidone.

The effects of sodium valproate and clonazepam on phenobarbitone, primidone and phenytoin serum concentrations will be discussed in the following chapter.

Variations in response to sodium valproate therapy between different patients did not appear to be related to variations in serum level of the drug. Most patients in the trial showed serum levels of sodium valproate in the range of 350-700  $\mu\text{mol/l}$  and other workers have found that/...

that the therapeutic levels lie within this range (Barnes and Bower, 1975). By comparison, serum levels of clonazepam showed wide variation between different individuals. Administration of other anticonvulsants lowered the serum level of clonazepam and hence presumably the effectiveness of the drug. This corresponds with the previous findings of my colleagues who collaborated with me in this work (Nanda, Johnson, Keogh, Lambie and Melville, 1977), in which they found that patients with high serum levels of phenobarbitone tended to have low serum concentrations of clonazepam; patients in that trial who were not receiving high doses of enzyme-inducing drugs such as phenobarbitone or primidone showed higher serum levels and better therapeutic control.

It appears that clonazepam is generally a less effective anticonvulsant drug than sodium valproate when added to standard anticonvulsant therapy, but it may be of value when used as a single drug or with low doses of other drugs; as it was found to be effective in the treatment of photosensitive and myoclonic epilepsy and in patients not receiving multiple drug anticonvulsant therapy, it was possible to increase the dose of clonazepam up to 12 mg daily without toxic effects.

There is therefore objective evidence for the greater value of sodium valproate when added to the drug therapy of patients already on anticonvulsants, compared with/...

with clonazepam. As it will be shown in the next chapter, this effectiveness of sodium valproate is independent from its effect on other anticonvulsants metabolism.

## SUMMARY

1. A cross-over comparative study of sodium valproate and clonazepam in the treatment of 32 adult chronic epileptic patients on multiple drug therapy is described. Fifteen patients completed all parts of the trial; 12 patients completed only three months on sodium valproate and five patients completed only three months on clonazepam.
2. Both drugs significantly reduced minor seizures, more so with sodium valproate than with clonazepam treatment ( $P < 0.005$  and  $P < 0.02$  respectively). Major seizures were reduced with sodium valproate, but this reduction failed to reach significance.
3. Drowsiness and ataxia were the two main side effects and were much more marked with clonazepam requiring withdrawal of treatment in many patients.
4. Sodium valproate is a more useful anticonvulsant than clonazepam when given to patients on multiple drug therapy.



## CHAPTER 2

The effects of sodium valproate and clonazepam on the serum levels of phenytoin, phenobarbitone and primidone in patients on multiple anticonvulsant therapy.

## INTRODUCTION

The effectiveness, side effects and serum concentrations of sodium valproate and clonazepam were discussed in Chapter 1. However, another important question as regards their interactions with three commonly used anticonvulsant drugs, viz phenytoin, phenobarbitone and primidone, needs to be answered. For example, serum phenobarbitone concentrations were reported to be increased by sodium valproate (Richens and Ahmed, 1975). It is also important to see whether or not the effects of control seen with each of the trial drugs is secondary to elevation of the serum levels of another drug.

## METHODS

Thirty-two adult chronic epileptic patients who were reported upon in Chapter 1 were studied and their serum phenytoin, phenobarbitone and primidone levels were monitored at four-weekly intervals to try to keep the/...

the serum concentrations of phenytoin, phenobarbitone and primidone within 15% of the base line in either direction.

Serum phenytoin, phenobarbitone and primidone were measured by Gas Chromatography (see under METHODS).

The differences in serum concentrations of anti-convulsants were compared using the Wilcoxon signed-ranks test.

### RESULTS

Seven patients showed an increase greater than 15% in serum phenobarbitone concentration when sodium valproate was added to their treatment. Five were receiving phenobarbitone and two were receiving primidone. Six of these patients showed side effects of drowsiness and ataxia. In all these patients, the dose of phenobarbitone or primidone was reduced. One of these patients also showed an increase in serum phenytoin concentration and in this case the phenytoin dose was reduced.

The phenobarbitone serum concentrations in the patients receiving phenobarbitone itself were significantly raised ( $P < 0.05$ , Wilcoxon signed-ranks test), with the mean increasing from  $142 \pm 25$  SD  $\mu\text{mol/l}$  to  $184 \pm 75$  SD  $\mu\text{mol/l}$  on administration of sodium valproate; the/...

the serum concentrations of phenobarbitone in patients receiving primidone were generally unchanged. There was a significant fall ( $P < 0.05$ ) in serum phenytoin concentrations with sodium valproate therapy (Fig. 2.1).

No significant effects on serum levels of phenytoin, phenobarbitone or primidone were observed with clonazepam therapy.

### DISCUSSION

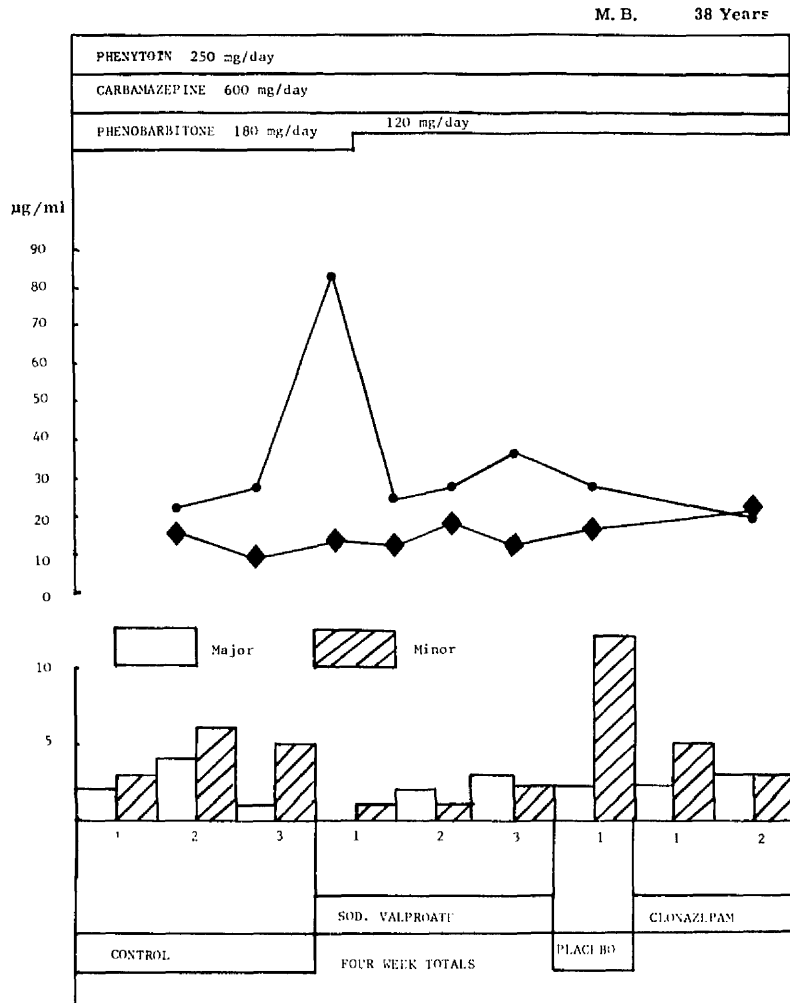
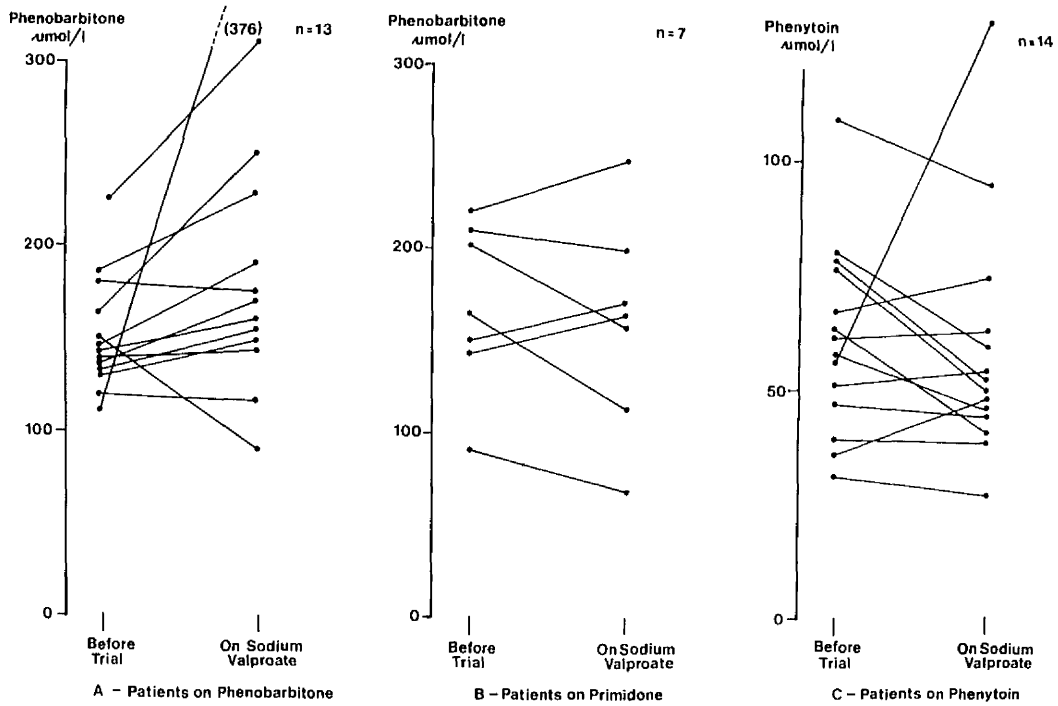
Overall, sodium valproate administration to patients on other anticonvulsants produced a significant increase in mean serum phenobarbitone concentration in patients receiving phenobarbitone, but not in patients receiving primidone. The mechanism of the effect of sodium valproate on serum levels of phenobarbitone is unknown, but the most likely hypothesis is that the drug inhibits metabolism of phenobarbitone. The apparent lack of effect of sodium valproate on phenobarbitone levels in patients receiving primidone might be explained by an inhibition by sodium valproate of primidone metabolism to phenobarbitone. Since the phenobarbitone or primidone dose was decreased in patients in whom serum levels of phenobarbitone significantly increased, it is likely that most of the therapeutic benefit obtained in patients during sodium valproate therapy was due/...

due to the action of the drug itself. However, evaluation of the drug in individual patients should take the possibility of drug interactions into account. For example, Fig. 2.2 shows serum drug concentrations and seizure control for a patient in whom the phenobarbitone serum level showed a dramatic increase on sodium valproate administration; the period before the phenobarbitone dose was decreased showed the lowest seizure frequency. However, the period after that the patient's seizure frequency was still less than the observation period and the drowsiness which appeared after the introduction of sodium valproate disappeared after reduction of phenobarbitone dose.

A significant decrease in serum levels of phenytoin was found on sodium valproate administration. This effect has been observed by other workers and it may be due to a displacement of phenytoin from its plasma protein binding sites (Patsalos and Lascalles, 1977). However, one patient showed a large increase in the phenytoin serum level which required dose reduction; this patient showed a concurrent increase in the phenobarbitone serum level and it is likely that this may have caused inhibition of phenytoin metabolism (Lambie, Johnson, Nanda and Shakir, 1976). A displacement of phenytoin from its protein binding sites may increase brain concentrations (Patsalos and Lascalles, 1977); further/...

Fig. 2.1 Serum levels of phenytoin, phenobarbitone and primidone in  $\mu\text{mol/l}$  before and during treatment with sodium valproate. Showing a significant increase in phenobarbitone ( $P < 0.05$ ) and a significant decrease in phenytoin ( $P < 0.05$ ) (using Wilcoxon signed-ranks test).

Fig. 2.2 Serum levels of phenobarbitone ( $\odot$ ), phenytoin ( $\circ$ ), and frequency of major ( $\square$ ) and minor ( $\boxtimes$ ) seizures in one patient before and after addition of sodium valproate to the treatment. The phenobarbitone level increased, but when the dose was reduced the improvement in seizure frequency remained.



further study of this as a possible factor involved in the therapeutic effect of sodium valproate would be desirable.

Variations in response to sodium valproate therapy between different patients did not appear to be related to variations in serum levels of the drug.

## SUMMARY

When sodium valproate or clonazepam were added to the anticonvulsant regimes of 32 adult chronic uncontrolled epileptic patients in a cross-over pattern

- (1) serum concentrations of phenytoin, phenobarbitone and primidone were unchanged when clonazepam was added;
- (2) with sodium valproate, serum phenobarbitone concentrations increased ( $P < 0.05$ ) in patients on phenobarbitone, but not significantly in patients on primidone;
- (3) phenytoin concentrations were reduced ( $P < 0.05$ ) during treatment with sodium valproate.



## SECTION II

This section deals with some of the effects that anticonvulsants have on the metabolism. The two effects that I studied were those on immunoglobulin and folate metabolism.

## CHAPTER 3

## Serum IgA depletion in epilepsy

## INTRODUCTION

Various immunological abnormalities have been reported during anticonvulsant therapy, including depression of both humeral and cellular immunity, in particular low serum immunoglobulin A (IgA) (Sorrell, Forbes, Burness and Rischbieth, 1971; Grob and Herold, 1972). Serum IgA depletion was not thought to be related to phenytoin therapy and was associated with higher incidence of psychiatric symptoms (Slavin, Fenton, Laundry and Reynolds, 1974). It was suggested that the IgA depletion was present in epileptic patients before the commencement of phenytoin therapy which accentuates it, and the deranged immunological finding may have some role in the aetiology of epilepsy (Seager, Jamison, Wilson, Hayward and Soothill, 1975).

## METHODS

Eighty-three epileptic patients were studied (40 females and 43 males) whose ages ranged between 17 and 76 years with a mean of 41 years. The patients were divided into two groups: Group A consisted of 56 patients, 24/...

24 female and 32 male, who were taking phenytoin in addition to other anticonvulsants; and Group B consisted of 27 patients, 15 female and 12 male, who were on anticonvulsants other than phenytoin. The duration of treatment was more than 10 years in all patients; the drug regimes of all the patients are shown in Table 3.1. Seven patients in Group A and four in Group B had family history of epilepsy. There was no patient included who suffered from neoplasia or any of the presumed auto-immune diseases.

Serum immunoglobulins: IgG, IgA and IgM were measured simultaneously by radial immunodiffusion (R.I.D.) and by the Technicon A.I.P. system.

Serum anticonvulsants: serum phenytoin, phenobarbitone and primidone were measured by gas chromatography.

Serum folates: measured by the Lacto-bacillus Casei method (for all assay techniques, see under METHODS).

## RESULTS

### Serum immunoglobulins

The 100% mean normal adult value (M.N.A.) was as follows:- IgG - 947 mg/dl, IgA - 248 mg/dl, IgM - 94 mg/dl, and the normal range for each was 50-175% M.N.A.

(Fahey and McKelvey, 1965).

In/...

DRUGS	Group 1 (56)	Group 2 (27)
Phenytoin	56	-
Phenobarbitone	29	11
Primidone	18	16
Carbamazepine	8	8
Sodium valproate	9	3
Clonazepam	1	1
Ethotoin	-	2

Table 3.1 : Group A - 56 patients all on phenytoin  
Group B - 27 patients on other anti-convulsants

In both groups, normal levels of IgG and IgM were found. With regard to IgA, however, a conspicuous difference was seen between Groups A and B (Fig. 3.1). In Group A, 13 patients (23.2%) had a low serum IgA concentration: less than 50% M.N.A., while in Group B only one patient had a slightly reduced IgA level (46% M.N.A.). The findings were consistent and when they were repeated after a month in 21 of the patients, while maintaining the same anticonvulsant dosage, the results showed a very good correlation (Fig. 3.2) ( $r = 0.962$ ;  $P < 0.001$ ). The two methods used for the assay of the immunoglobulins, i.e. R.I.D. and nephelometry, again showed consistent results ( $r = 0.894$ ;  $P < 0.001$ ) (Fig. 3.3).

#### Serum anticonvulsants

No correlation was found between the dose or the serum concentration of phenytoin and the degree of IgA depletion. In Group A, however, patients who had low serum IgA levels had lower serum primidone levels than patients with normal serum IgA, while the serum phenobarbitone derived from primidone was not different in the two groups. Four of the patients with low IgA were on phenobarbitone and these had a high serum level when compared with those with normal serum IgA in Group A and patients in Group B in spite of the fact that they were on comparable doses (Table 3.2). The primidone and the/...

Fig. 3.1 Serum IgA mg/dl in 56 patients on phenytoin, and 27 patients on anticonvulsants other than phenytoin. Thirteen patients in the first group had levels below 50% Mean Normal Adult (M.N.A.). Only one patient in the second group had a slightly reduced level.

Patients on  
Phenytoin

Patients on  
other drugs

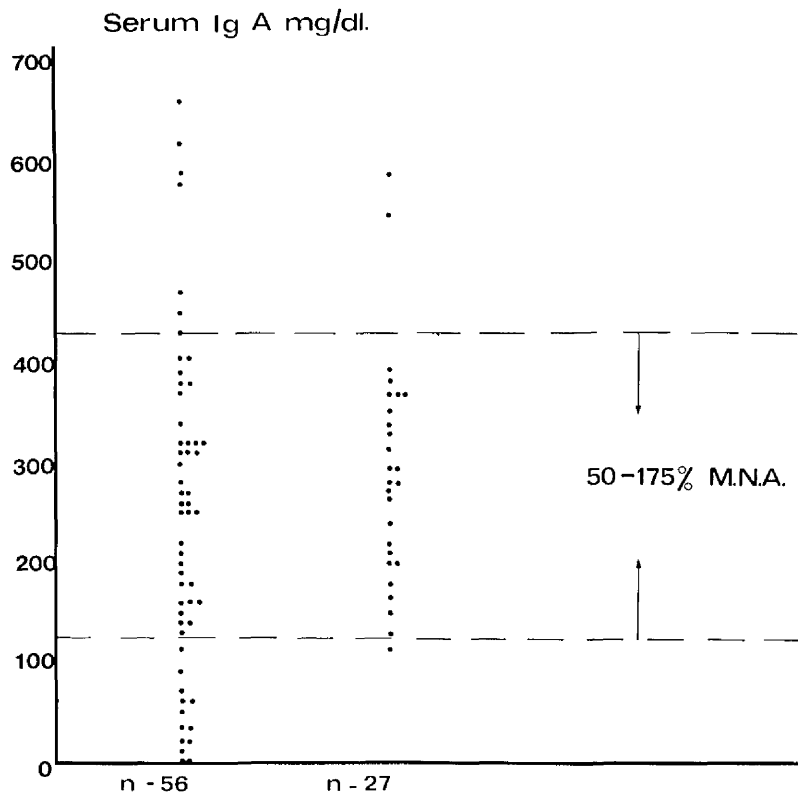
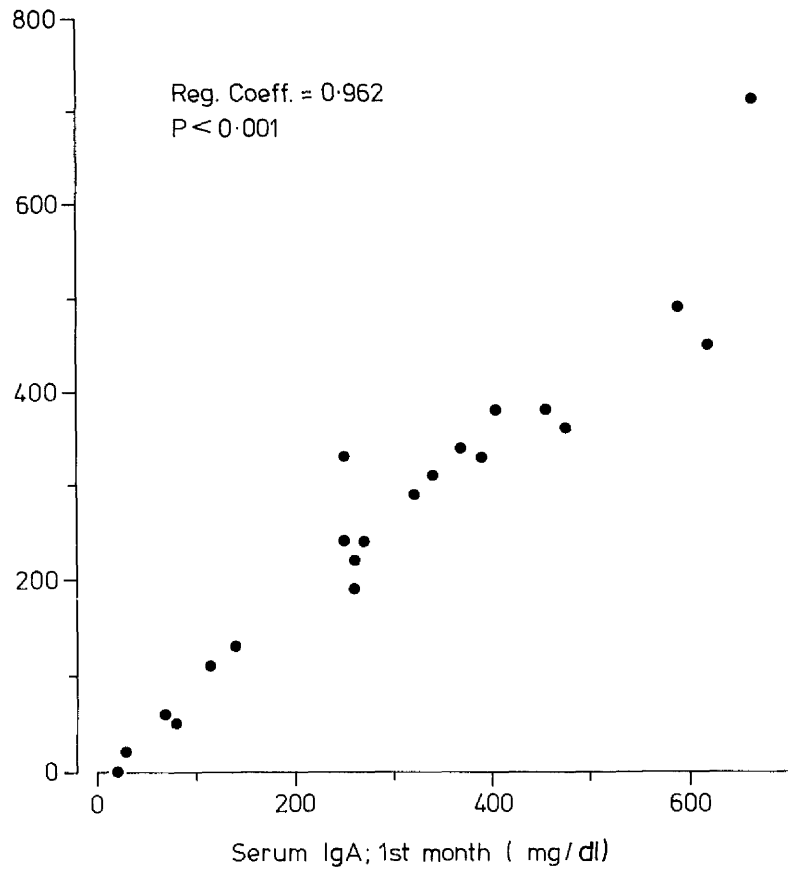


Fig. 3.2 Serum IgA in mg/dl in 21 patients on phenytoin.  
Correlation of levels done on two occasions.  
 $r = 0.962$ ,  $P < 0.001$ .

Fig. 3.3 Serum IgA in mg/dl. Correlation between radial  
immunodiffusion and nephelometry in 21 patients  
on phenytoin.  $r = 0.894$ ,  $P < 0.001$ .

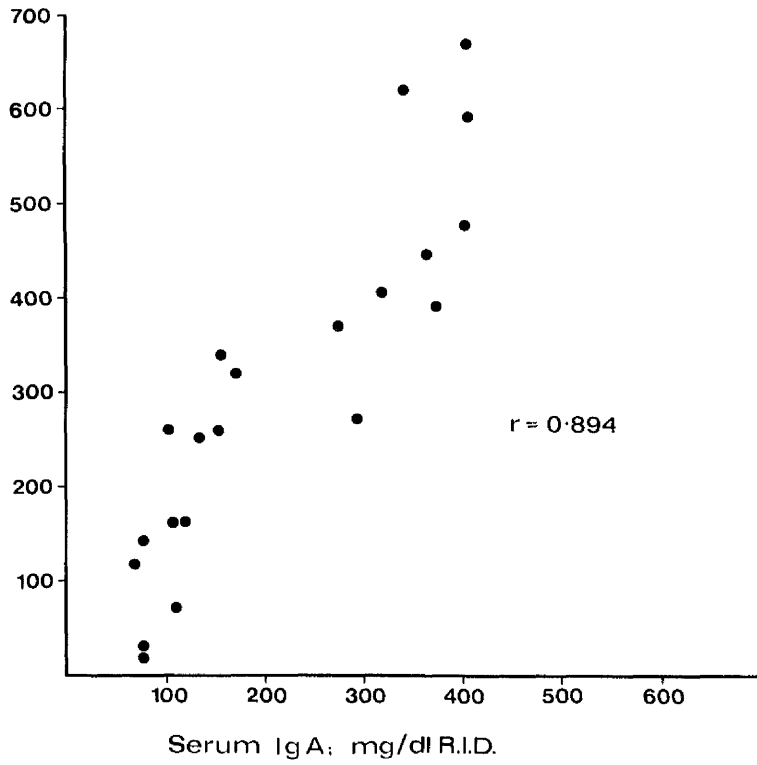


Serum IgA  
2nd month  
( mg/ dl)



### IgA Results Consistency

Serum IgA mg./dl  
A.I.P. System



Drugs	Patients on phenytoin (Group A)				On other drugs (Group B)	
	No.	Low serum Iga	No.	Normal serum Iga	No.	
Phenytoin	13	43.23 ± 23.58 SD	43	41.6 ± 25.6 SD	-	-
Phenobarbitone	4	179.25 ± 63.24 SD	24	118.86 ± 45.6 SD	10	111.8 ± 52.31 SD
Primidone	7	17.14 ± 8.68 SD	16	32.75 ± 19.1 SD	15	23.5 ± 9 SD
(Phenobarbitone)*	7	123 ± 42.19 SD	16	120.1 ± 58 SD	15	118 ± 60.4 SD

\* Phenobarbitone derived from primidone

Table 3.2 : Serum anticonvulsant levels in  $\mu\text{mol/l}$  in the two groups of patients.

the phenobarbitone levels were significantly different ( $P < 0.05$  for both using Student's  $t$  test) in the two groups of patients, but because of the small number of patients, this finding is not considered to be important.

#### Serum folate levels

Low serum folate levels were found in both Groups A and B ( $2.22 \mu\text{g/l} \pm 0.95 \text{ SD}$  and  $3.164 \mu\text{g/l} \pm 1.567 \text{ SD}$ , respectively). The normal range of serum folate by the method used is  $7 - 15.9 \mu\text{g/l}$  and the lowest limit of the intermediate range is  $3 \mu\text{g/l}$  (Herbert, 1966). There was a significant difference between Groups A and B, patients in Group A having lower serum folate levels ( $P < 0.0005$  using Student's  $t$  test). There was no correlation between the serum folate levels and the IgA deficiency. Patients with low serum IgA had a mean serum folate of  $2.55 \mu\text{g/l} \pm 1.003 \text{ SD}$ .

There was no correlation between the type of epilepsy, seizure control or the incidence of psychiatric symptoms and the serum IgA levels. Only three patients of the 13 with low serum IgA had a positive family history of epilepsy.

#### DISCUSSION

IgA deficiency is the most commonly reported immunologic defect in man. One in 500 to 800 persons have/...

have selective IgA deficiency (Koistinen, 1975). Not all of these people manifest disease. Therefore additional factors appear to contribute to the ultimate expression of disease.

There is good evidence that immunological abnormalities are found in patients taking anticonvulsants. Several workers have found low serum IgA levels and evidence of impaired T-cell function (Sorrell and Forbes, 1975). The mechanisms involved in producing the immune changes are unknown.

Nearly one-fourth of the patients (23.2%) who were on phenytoin had low serum IgA levels, while only one patient from Group B had even a slightly reduced level, and it is interesting to note that this patient was on carbamazepine, which was reported to cause IgA depletion in 11% of patients (Sorrell and Forbes, 1975). In the patients affected, there was no relationship between phenytoin dosage or serum level and the degree of IgA depression. The reduced serum primidone levels which were found in patients with low serum IgA have been reported previously (Slavin et al., 1974), but the explanation of this is unknown.

IgA function is related to intact thymic function (Lancet, 1975). It is known that congenitally athymic nude mice have IgA deficiency although they have many IgA/...

IgA bearing B lymphocytes (Bankhurst and Warner, 1972). This is similar to patients with IgA deficiency secondary to phenytoin therapy (Sorrell and Forbes, 1975), who also have normal numbers of IgA bearing B lymphocytes, but low serum IgA levels. In animal experiments impaired immune responses, atrophy of thymic and lymphoid tissue and increased incidence of lymphoid tumours were produced by chronic phenytoin ingestion (Kruger and Harris, 1972), and indeed increased incidence of malignant lymphomas associated with phenytoin therapy in epileptic patients has been reported (Anthony, 1970).

The mechanism of production of low serum IgA by phenytoin is not yet understood. A direct action of the phenytoin on the plasma cell situated in the submucous layers mainly of the gut and the respiratory system is possible, but there must be another factor which predisposes some patients to develop the deficiency as long as it is not dose or serum level related. This factor was suggested to be a constitutional one, and this will be investigated in Chapter 5. Another factor which might be operative is the folate deficiency which may limit protein synthesis (Norris and Pratt, 1974). The incidence and the effects of folate deficiency will be discussed in later chapters of this thesis.

Reduced serum IgA has been found in patients with a variety of auto-immune disorders, including systemic lupus erythematosus/...

erythematosis (Tomasi, 1968), pernicious anaemia (Odgers and Wagerl, 1968), Sjögren's syndrome (Claman, Hartley and Merrill, 1966; Hobbs, 1968), and myasthenia gravis (Simpson, Behan and Dick, 1976), and in a variety of malignancies (Scheurlen, Schneider and Pappas, 1971).

In the patients with low serum IgA level, the incidence of psychiatric symptoms was not more than in those with normal levels and all of them were on phenytoin, a finding which does not support the results of Slavin et al. (1974) who have suggested that the IgA deficiency is not related to anticonvulsant therapy and especially phenytoin, and who have reported an increased incidence of psychiatric symptoms in the low IgA group of their patients. The abnormality of reduced serum IgA during phenytoin therapy was reported to be of a greater extent in female patients, with depression of IgM levels (Sorrell and Forbes, 1975). However, the findings in this study were that the 13 patients with low IgA showed a male:female ratio of 9:4, and the serum IgG and IgM were not different in the low IgA group from other patients and indeed they were within the normal limit, which is not in agreement with other workers (Slavin et al., 1974).

Patients with low serum IgA did not have an increased liability to infection as would be expected in some of them (South, Cooper, Wollheim and Good, 1968). This really/...

really depends on the state of the secretory IgA in their mucosal secretions; although this has been reported to be reduced (Aarli and Tønder, 1975), absence of increased liability of sino-pulmonary or other infections does not seem to support this. In addition, the low salivary IgA was postulated to be a contributing factor in the development of gingival hyperplasia which is a common complication of phenytoin therapy. However, other workers (Harris and Rowe, 1976), in examining 103 epileptic patients, showed a 65% incidence of gingival hyperplasia and its degree was correlated with high serum concentrations of alkaline phosphatase. In view of these reports, salivary IgA was studied, details of which are in the following chapter.

## SUMMARY

1. Serum IgA was found to be reduced in 23.2% of 56 patients taking phenytoin as part of their anticonvulsant medication and only slightly reduced in one of 27 patients on anticonvulsants other than phenytoin.
2. The IgA deficiency was not related to the dose or the serum concentration of phenytoin.
3. No difference in the serum IgG or IgM was noted between the two groups.
4. Serum folate was low in both groups, but the reduction was more in the group that was on phenytoin and the difference was statistically significant.
5. There was no correlation between the serum IgA level and the seizure control or duration of the disease, and there was no specific liability to infection in the group who had low serum IgA levels.



## CHAPTER 4

## Salivary IgA in patients on phenytoin

## INTRODUCTION

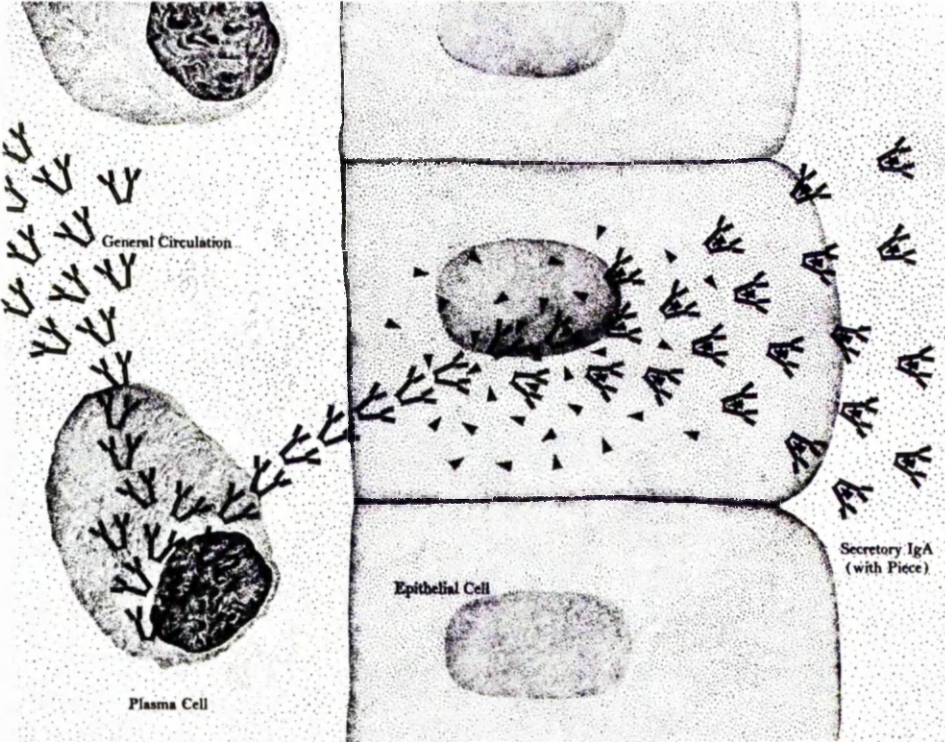
The plasma cells present in the submucous layer of the respiratory, gastro-intestinal and urinary tracts produce IgA which has the function of acting as an antibody on these mucosal surfaces (Fig. 4.1). The IgA molecule passes through the epithelial lining of the mucosa and during its passage two IgA molecules are attached together with another substance called the secretory piece; the product is called secretory IgA which is resistant to the action of proteolytic enzymes (Tomasi, 1971). The production of secretory IgA into external secretion is controlled independently of systemic immunological reactions and the distribution of secretory IgA differs strikingly from that of the serum (Walker and Isselbacher, 1977).

One way of looking at the secretory IgA is by measuring its concentrations in saliva. In a recent study in epileptic patients who were on phenytoin, with low serum IgA concentrations, the secretory IgA was shown to be reduced in whole saliva and this was correlated with gum hyperplasia (Aarli and Tønder, 1975).

In Chapter 3 of this thesis, the patients who were found/...

Fig. 4.1 Synthesis and transport of secretory IgA.  
(Tomasi, 1971). Plasma cell in the submucous  
layer secretes IgA which goes through the  
mucosal layer, where two molecules are attached  
together by the secretory piece.

Synthesis and Transport of Secretory IgA



found to have low serum IgA concentrations did not show an increased incidence of sinopulmonary infections, and gingival hyperplasia was not confined to them. Gingival hyperplasia has been correlated with high serum concentrations of bone alkaline phosphatase (Harris and Rowe, 1976). In addition, the patients with low serum IgA did not show an increased liability to infections as would be expected in patients with reduced IgA on their epithelial surfaces (Tomasi, 1971).

#### PATIENTS and METHODS

Seven epileptic patients with low serum IgA concentrations, six other epileptic patients with normal serum IgA, and 10 healthy control adults were studied. The two groups of epileptic patients were taking phenytoin in addition to other anticonvulsants (Table 4.1).

In both groups of patients, the serum IgA concentrations were measured on three occasions, except in three patients where it was done twice.

Saliva was collected from one or the other parotid duct opening using Carlson-Crittenden cups. Both salivary and serum IgA were measured by the A.I.P. system.

Serum anticonvulsants - phenytoin, phenobarbitone and primidone - were measured by Gas Chromatography (for details of the techniques see METHODS).

RESULTS/...

DRUGS	Low Serum IgA	Normal Serum IgA
Phenytoin	7	6
Phenobarbitone	3	4
Primidone	3	2
Carbamazepine	2	1
Sodium Valproate	-	1

Table 4.1 : Drug therapy in the patients examined

## RESULTS

Tables 4.2 and 4.3 show the serum IgA concentrations in mg/dl with their means, and the % Mean Normal Adult value of the means, and it is very evident that in the two groups there is a big difference between the serum IgA concentrations. The salivary IgA concentration results are shown in Table 4.4 in both groups of patients and in the controls. Trying to look at the secretory IgA as part of the IgA system, the salivary value is expressed as a percentage of the serum level, although in absolute terms the salivary IgA concentration was lowest in the patients with low serum IgA concentrations (statistically not significant), but the percentage of salivary to serum concentrations was the highest, a mean of 40.7% compared to the controls mean of 10.9% ( $P < 0.01$  using Student's  $t$  test).

Serum anticonvulsant concentrations for both groups of patients are shown in Table 4.5, and it is evident that both groups of patients were under adequate dosage of anticonvulsants. The serum IgA depletion was not related to the serum phenytoin level, as the low IgA group had a lower mean serum phenytoin concentration.

## DISCUSSION

The presence of reduced serum IgA concentration does not/...

Patients with low serum IgA

Patients	Serum IgA mg/dl			Mean	% M.N.A.
	First	Second	Third		
1	70	60	100	76	30.6
2	115	110	90	105	42.3
3	0	-	20	10	4
4	20	0	45	21.6	8.7
5	90	-	95	92.5	37.2
6	35	-	30	32.5	13.1
7	30	20	5	18.3	7.3

Table 4.2 : Serum IgA levels on three occasions in four patients and two occasions in three patients, in mg/dl, with the mean and the mean normal adult percentage (M.N.A.%)

Patients with normal serum IgA

Patients	Serum IgA mg/dl			Mean	% M.N.A.
	First	Second	Third		
1	475	360	430	421	169
2	390	330	380	366	147
3	340	310	210	286	115
4	370	340	310	340	137
5	270	240	180	230	92
6	405	380	270	351	141

Table 4.3 : Serum IgA levels on three occasions in mg/dl with the mean and the mean normal adult percentage (M.N.A.%)



Salivary IgA mg/dl

Low serum IgA group (n=7)			Normal serum IgA group (N=6)			Controls (n=10)		
No.	Parotid saliva	% of serum levels	No.	Parotid saliva	% of serum levels	No.	Parotid saliva	% of serum levels
1	25	32.8	1	23	5.4	1	24.2	17.9
2	10	9.5	2	8	2.1	2	24.2	9.6
3	4	40	3	4	1.3	3	6.7	5.5
4	21.6	78	4	54	15.8	4	27.5	39.2
5	13	14	5	46	20	5	9.2	5.9
6	6	18.4	6	13	3.7	6	17.1	4.5
7	17	92.8				7	1.7	0.89
						8	22.9	9.5
						9	4.9	7.6
						10	12.5	8.9

Means 13.1 ± 7.2SD      40.7% ± 32.5SD      24.6 ± 20.7SD      8% ± 7.8SD      15 ± 9.3SD      10.9% ± 10.8SD

Table 4.4 : Salivary IgA concentrations in mg/dl in the two groups of patients and controls with % of the salivary to the serum IgA levels.

Serum anticonvulsants  $\mu\text{mol/l}$

Patients	Patients with low serum IgA			Patients with normal serum IgA			
	Phenytoin	Pheno-barbitone	Primidone	Patients	Phenytoin	Pheno-barbitone	Primidone
1	77	117	-	1	92	73	-
2	30	169	-	2	38	73	-
3	52	128	-	3	42	73	11
4	13	-	-	4	102	112	43
5	32	140	8	5	74	117	-
6	46	182	18	6	21	113	-
7	23	116	26				

Means:- 39  $\pm$  21.3 142  $\pm$  27.6 17.3  $\pm$  9 61.5  $\pm$  32.5 93.5  $\pm$  22.5 27  $\pm$  22.6

Table 4.5 : The serum phenytoin, phenobarbitone and primidone in both groups of patients.

not necessarily indicate a similar reduction of secretory IgA. Indeed, there is little consistent relationship between secretory and serum IgA levels, and there are several infants reported with significant amounts of secretory IgA, but no detectable serum IgA (Tomasi, 1971). The other fact that stimulated this work was that the patients with low serum IgA did not show an increased incidence of infection, particularly of the respiratory tract.

Salivary IgA was reported to be reduced in patients on phenytoin and was correlated with gum hypertrophy (Aarli and Tønder, 1975). On the other hand, gum hyperplasia was correlated with high serum alkaline phosphatase in 65% of 103 patients (Harris and Rowe, 1976). In the patients studied here the degree of gum hypertrophy was not different or more pronounced in the patients who had low serum IgA.

An important finding as regards the level of salivary IgA in controls is noted. Previous reports on parotid saliva IgA concentrations in unstimulated healthy adults have shown a mean of 4.5 mg/dl (Oon and Lee, 1972), while in mixed saliva the mean secretory IgA was reported as between 3.2 and 4.5% of the serum levels in 10 healthy adults (Aarli and Tønder, 1975). In both studies, radial immunodiffusion, using low concentration Agar plates, was the method by which salivary/...

salivary IgA levels were measured. In this study, parotid saliva was collected unstimulated to avoid the dilution effect which had been reported previously (Oon and Lee, 1972), and nephelometry was used to measure the concentration of the immunoglobulins. This may have led to the higher salivary IgA concentrations because the number of molecules and not the speed of diffusion of the larger secretory IIS particles was measured. In both studies mentioned above and indeed in this one, serum IgA was used as a standard. Another point worth mentioning here is the great fluctuation in parotid salivary IgA concentration when measured at weekly intervals by electroimmunodiffusion and the values in the controls in this study certainly fall within the range of normal fluctuation (Claman, Merrill and Hartley, 1967). Another finding stated by the last mentioned authors was the lower secretory IgA found in submandibular-sublingual saliva than in parotid saliva, which would explain a lower IgA in whole saliva when compared to parotid secretory IgA.

The finding of a higher percentage of salivary IgA when compared to the serum level in this study would point to a possibility that there is some effect on the serum IgA, possibly excessive destruction, or more likely perhaps that there is a direct effect on the plasma cell. This may have led to partial, but not complete, impairment of function, and whatever IgA produced is preferentially/...

entially thrown out through the mucosal layer to become the secretory IgA, which is after all the functional IgA in the body. This may therefore lead to a reduction in the serum IgA level. The local plasma cell is the important factor in the function of the secretory IgA. This important local effect has been demonstrated in another way, by introducing inactivated polio virus into double-barreled colostomies, the highest IgA antiviral antibody levels were obtained from sites in direct contact with the virus. This was not seen in sites distant from the site of introduction (Tomasi, 1976).

There was no relation between the salivary IgA concentration and the dose or serum level of phenytoin, and this is in accordance with the findings in Chapter 3 of this thesis.

In conclusion, the secretory IgA seems to be affected to a much lesser degree than the serum IgA in epileptic patients who are on long-term anticonvulsant medication, indicating perhaps a partial or incomplete inhibitory effect on the plasma cell present in the submucous layers of the gastro-intestinal and respiratory tracts.

IgA deficiency will be studied further in the next chapter as regards correlation with the HLA system and possible constitutional predisposition to IgA deficiency in patients who take phenytoin.

## SUMMARY

1. Secretory IgA was not found to be depressed to the same degree as serum IgA in patients on long-term phenytoin therapy.
2. Gum hypertrophy, a common side effect of chronic phenytoin intake, was not related to reduced salivary IgA.
3. The method by which phenytoin affects serum IgA level might be through partial inhibition of the plasma cell in the submucous layers of epithelial covers of the gastro-intestinal and respiratory tracts.
4. Unstimulated parotid saliva may be a better way of studying secretory IgA than mixed saliva.

## CHAPTER 5

Metabolism of immunoglobulin A, lymphocyte function and histocompatibility antigens in patients on anticonvulsants.

## INTRODUCTION

As discussed in the previous two chapters, serum IgA levels were found to be depressed in roughly 25% of patients who are on long-term phenytoin therapy. Cell-mediated immunity has been measured in vivo by skin testing with ubiquitous recall antigens, and in vitro by measurement of lymphocyte DNA and RNA synthesis. It was found to be depressed; low serum IgA levels together with defective antibody production to Salmonella typhi and tetanus toxoid were also found (Sorrell and Forbes, 1975). It was subsequently suggested that epilepsy with constitutional characteristics might predispose to low IgA and that the low IgA only occurs when phenytoin is given (Fontana et al., 1976).

## PATIENTS and METHODS

From the 83 patients that were studied in Chapter 3 of this thesis, 30 patients were selected for this study. Ten patients were taking phenytoin as part of their/...

their anticonvulsant medication and were shown to have consistently low serum IgA levels (below 50% M.N.A.). Ten other patients were having phenytoin as well, but had consistently normal serum IgA levels. The last group of patients were 10 patients who were on anti-convulsant drugs other than phenytoin (including phenobarbitone, primidone, carbamazepine, and sodium valproate) and had normal serum IgA levels.

#### Peripheral blood lymphocyte protein synthesis

The synthesis of protein by peripheral blood lymphocytes was measured by the whole blood technique (Behan, Behan, Zacharias and Nicholls, 1976). This method estimates the uptake of tritiated leucine by peripheral blood lymphocytes on stimulation with purified phyto-haemagglutinin (PHA) over a period of 22 hours. Lymphocytes from the 30 patients described above were studied. A dose response curve to various concentrations of purified PHA (Burroughs Wellcome) was drawn from each patient. Protein synthesis by lymphocytes was also measured in 35 healthy control adults.

#### HLA typing

Twenty of the 30 patients mentioned earlier were typed for their histocompatibility antigens (HLA). These were the patients who were taking phenytoin, half of them had low serum IgA and the other half had normal serum/...



serum IgA levels. HLA typing was carried out by a modification (Dick, Crichton, Ferguson-Smith and Izatt, 1972) of the lymphocytotoxicity testing using glass plates, described by Kissmyer-Nielsen and Kjerbye (1967).

## RESULTS

### HLA typing

The HLA pattern is shown in Table 5.1. There were eight patients with HLA-A<sub>2</sub> in those with low serum IgA compared to three in those with normal serum IgA.

### Lymphocyte protein synthesis

Optimum stimulation was obtained at a PHA concentration of 8.33 µg/ml. Responses to PHA are expressed as the ratio of the counts/minute with PHA to counts/minute in the unstimulated cultures. A highly significant depression of protein synthesis was found in all 30 patients when compared to the controls (Fig 5.1).

## DISCUSSION

There is good evidence that immunological abnormalities are found in patients taking anticonvulsants. It was demonstrated in the previous two chapters that phenytoin affects the IgA producing plasma cells and indeed other workers have shown evidence of impaired T-cell/...

Fig. 5.1 Lymphocyte protein synthesis in response to phyto-haemagglutinin (PHA) at a concentration of 8.33  $\mu\text{g}/\text{ml}$ .

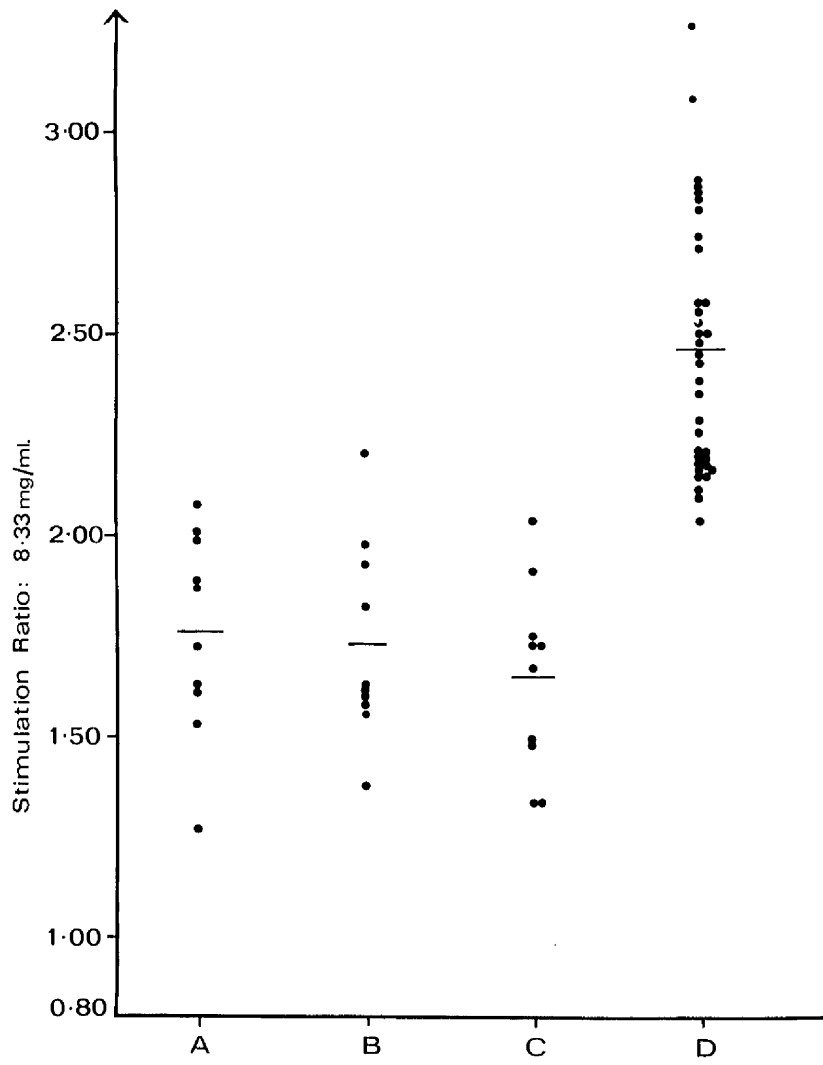
A : Patients with low IgA on phenytoin and other drugs ( $P < 0.0001$ ).

B : Patients with normal IgA on phenytoin and other drugs ( $P < 0.0001$ ).

C : Patients with normal IgA on other drugs ( $P < 0.0001$ ).

D : Normal controls (35).

Differences were assessed using Student's t test.



Patients	HLA-A		HLA-B	
Low serum IgA group				
male	2	3	7	W17
female	2	28	8	14
male	2	3	27	W16
male	2	W19	14	W21
female	2		7	13
male	2	3	5	W15
male	2	9	7	W16
male	2	9	5	7
female	3	W26	27	W40
male	3	9	7	12
Normal serum IgA group				
male	2	3	7	W14
female	2	11	12	W37
male	2	W25	W40	
female	1	9	7	8
female	1	2	8	W15
female	1		8	W17
male	1	29	W17	
male	3	W32	7	W35
female	3	W32	8	W27
male	3		7	

Table 5.1 : HLA typing in 20 patients on phenytoin.

T-cell function as measured both in vivo by lack of cutaneous reactivity to ubiquitous recall antigens, and in vitro by impaired lymphocyte DNA and RNA synthesis (Sorrell and Forbes, 1975). The mechanisms involved in producing these immune deficiencies are unknown, but it has been suggested that a genetic factor may be operative (Fontana et al., 1976).

The results of this work show a conspicuous impairment of T-cell function. The technique used to measure T-cell function was the in vitro measurement of peripheral blood-lymphocyte protein synthesis. This technique has been demonstrated to be a sensitive and reliable index of thymus-derived lymphocyte function (Pauly, Sokal and Han, 1973). Impaired immune responses by this method have previously been demonstrated in patients with auto-immune diseases, drug reactions and malignancies (Thomas, Lannigan and Behan, 1975; Behan et al., 1976; Simpson, Behan and Dick, 1976).

It is possible that phenytoin causes depressed T-cell function by direct action on cells. This hypothesis is supported by evidence of the action of phenytoin on normal lymphocytes in culture, where it produces depression of both DNA and RNA synthesis (Sorrell and Forbes, 1975). However, in this study, patients who were taking anticonvulsants other than phenytoin had a similar depressed lymphocytic response. A factor common to/...

to both groups and demonstrated in Chapter 3 of this thesis was reduced serum folate levels; this is known to affect cellular nucleic acid synthesis (Norris and Pratt, 1974). This effect of anticonvulsants on folic acid metabolism will be the subject of study in the following chapter of this thesis.

The most interesting association was the increased frequency of the histocompatibility antigen HLA-A<sub>2</sub> in the patients taking phenytoin with low serum IgA levels. Although the sample size was small, the difference between the two groups was striking and strongly supports the hypothesis that phenytoin causes selective IgA depletion in genetically predisposed individuals.

## SUMMARY

1. Impaired T-cell function demonstrated by depressed protein synthesis of peripheral blood lymphocytes upon stimulation with PHA has been shown to be present in 30 epileptic patients on chronic anticonvulsant medication.
2. The impaired T-cell function was not related to any specific anticonvulsant, nor to patients who had reduced serum IgA as a result of phenytoin intake.
3. The effect on T-cell function could be due to a common factor perhaps folate deficiency secondary to anticonvulsant medication.
4. The patients with reduced serum IgA had a higher incidence of the histocompatibility antigen HLA-A<sub>2</sub> which was significantly higher than those patients who were on the same medication but had a normal serum IgA, suggesting a genetic predisposition to develop selective IgA deficiency after intake of phenytoin.

## CHAPTER 6

Serum and cerebrospinal fluid folate levels in epilepsy.

## INTRODUCTION

Reduced serum folate levels have previously been reported in a large percentage of patients taking anti-convulsant medication. This varied from 33-99% (Hawkins and Meynell, 1958; Klipstein, 1964; Malpas, Spray and Witts, 1966; Jensen and Olesen, 1970; Norris and Pratt, 1971; Reynolds, Mattson and Gallagher, 1972). In Chapter 3 of this thesis, low serum folate levels were noted in the patients examined. This was more pronounced in those who had phenytoin as part of their anticonvulsant medication. The cerebrospinal fluid folate concentration is normally higher than that of the serum (Wells and Casey, 1967), but CSF folate was also reported to be reduced in epileptic patients on anti-convulsants (Reynolds et al., 1972). In the following chapter of this thesis, the extent of folate deficiency, and the effects of treatment of folate deficient epileptics will be studied, and the patients in this chapter who had the lowest serum folate levels will be the subject of further study in the next chapter.

## METHODS

Fifty-two adult epileptic patients, age range 17 - 63/...



63, mean 38 years, were studied. They were 27 females and 25 males. All were on multiple anticonvulsant drug therapy: phenytoin 35 patients, phenobarbitone 25 patients, primidone 25 patients, in addition to carbamazepine, sodium valproate, clonazepam and ethotoin.

Serum folate measurements were done in all the patients. CSF folate was subsequently measured in 20 patients who had the lowest serum folate levels and in those 20 the serum folate was measured twice. CSF was obtained by lumbar puncture within one hour of awakening in the morning; blood was taken simultaneously for serum folate estimation. Serum and CSF folate were measured by the *Lactobacillus Casei* method (see under METHODS). Serum phenytoin, phenobarbitone and primidone were measured by Gas Chromatography. For correlating serum and CSF folate concentration, data were logarithmically transformed because the folates are log normally distributed (Reynolds, Gallagher, Mattson, Bowers and Johnson, 1972).

## RESULTS

Serum folate levels in all the patients had a mean of  $2 \mu\text{g/l} \pm 0.91 \text{ S.D.}$  Five patients only had a serum level above  $3 \mu\text{g/l}$  (90.3% had low serum folate). Of the 20 patients in whom lumbar punctures were performed to obtain CSF, only 17 patients had their CSF samples successfully/...

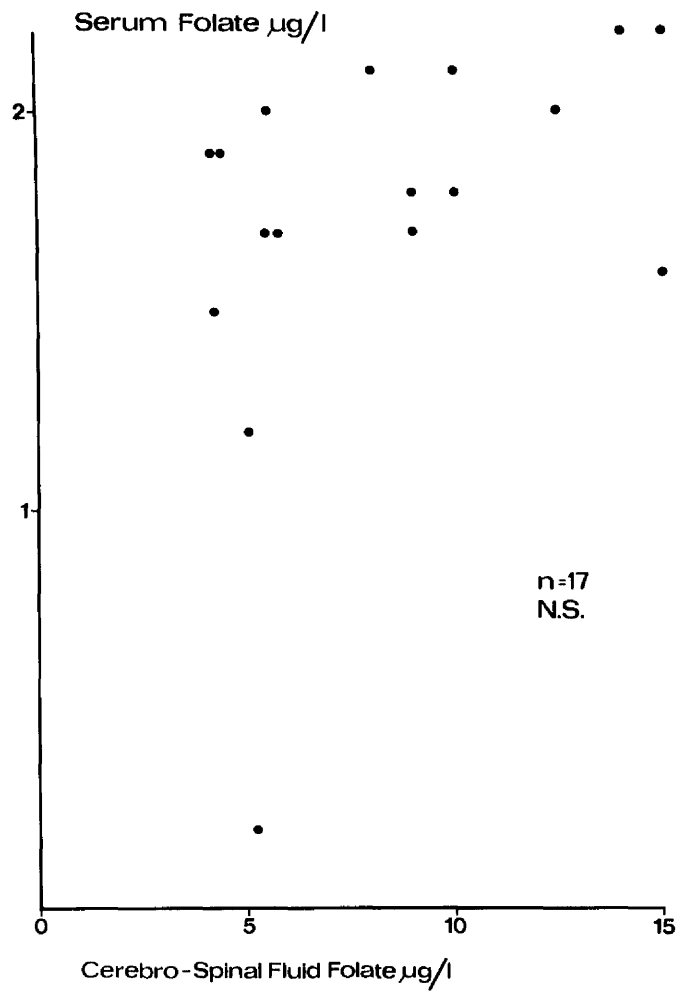
successfully obtained; the serum folate level in them was  $1.74 \mu\text{g}/\text{l} \pm 0.48 \text{ S.D.}$ , and the CSF folate levels had a mean of  $8.35 \mu\text{g}/\text{l} \pm 3.86 \text{ S.D.}$ , a ratio of serum : CSF of 1 : 4.8, but there was no significant correlation between the serum and the CSF folate levels in the group examined (Fig. 6.1).

Serum anticonvulsant concentrations: serum phenytoin levels had a mean of  $66.45 \mu\text{mol}/\text{l} \pm 27.88 \text{ S.D.}$ , serum phenobarbitone was  $146.68 \mu\text{mol}/\text{l} \pm 51.48 \text{ S.D.}$ , and serum primidone concentration was  $46.09 \mu\text{mol}/\text{l} \pm 21.02 \text{ S.D.}$  There was no significant correlation between the serum phenytoin and the serum folate concentrations, or indeed the phenobarbitone and primidone. The serum folate levels in patients taking phenytoin had a mean of  $1.78 \mu\text{g}/\text{l} \pm 0.45 \text{ S.D.}$ , compared with  $1.99 \pm 0.97 \mu\text{g}/\text{l}$  in patients who did not have phenytoin as part of their anticonvulsant medication and there was no significant difference between the two using Student's t test.

#### DISCUSSION

It seems that the group of epileptic patients studied were similar to what other workers have reported, and the low serum and CSF folate levels were almost universal. Folates are important in various aspects of intracellular metabolism, acting as a methyl donor, and are essential in nucleic acid synthesis (Norris and Pratt/...

Fig. 6.1 Correlation between serum and CSF folate  
 $\mu\text{g}/\text{l}$  in 17 patients. No significant  
correlation.



Pratt, 1974). In the patients examined, low serum folate was found in 90.3% of the patients and this is comparable to the findings of other workers, although a significantly lower serum folate was found in patients taking phenytoin as part of their anticonvulsant therapy than in those who were on other anticonvulsants (Chapter 3); in this study, the mean serum folate in patients on phenytoin was slightly lower, but this difference was not significant. The reason for this may be the larger group of patients examined in Chapter 3.

Folic acid has been shown to have convulsive properties on its own and antagonises the action of phenytoin in the rat (Hommes and Obbens, 1972). In addition, various reports were published on the effect of folic acid therapy on the seizure frequency and mental state of folate deficient epileptics. Worsening of seizure frequency was reported in 50% of 26 patients (Reynolds and Wales, 1967; Wells, 1968). However, more recent reports have not confirmed this finding and folate therapy was reported to have no significant effect on the seizure frequency or mental state of the patient (Jensen and Olesen, 1970; Mattson, Gallagher, Reynolds and Glass, 1973). In this study, the patients in whom CSF folate was measured showed a reduction in the CSF values when compared to previous reports on CSF folate levels in patients with other neurological disease (Wells/...

(Wells and Casey, 1967). The CSF folate did not correlate with serum folate as was reported previously (Wells and Casey, 1967) probably because the patients who had CSF folate examinations in this study were those with the lowest serum folate in the group and the differences between CSF folate levels were more than those of the serum. The serum anticonvulsants in the patients examined had means which were within the so-called therapeutic levels for each drug, indicating that compliance of the patients examined was quite satisfactory.

The cause of folate deficiency in patients on anti-convulsants is still unknown. However, an effect on the conversion of folic acid to 5-methyltetrahydrofolate has been hypothesised and it has been reported that CSF folate levels do not show a significant increase after folic acid therapy, but there is a significant increase when the reduced form of folic acid (5-formyltetrahydrofolate) is used (Mattson et al., 1973).

The effects of treatment of folate deficient epileptics with folic acid and 5-formyltetrahydrofolate will be discussed in the next chapter.

## SUMMARY

1. Fifty-two adult epileptic patients had their serum folate examined; in 20 of them who showed the lowest serum folate levels, lumbar punctures were performed and the CSF folate was estimated.
2. A large percentage (90%) had reduced serum folate levels. CSF folate was reduced as well and the serum : CSF ratio was 1 : 4.8.
3. Serum and CSF folate did not show a significant correlation.
4. The degree of reduction in serum folate was more in patients who were on, among other drugs, phenytoin, but the difference between them and those who were on drugs other than phenytoin was not statistically significant.

## CHAPTER 7

Effects of treatment with folic acid or 5-formyl-tetrahydrofolate on serum and CSF folate and on CSF amine metabolites in epileptic patients.

## INTRODUCTION

Treatment of epileptic patients who have low serum and CSF folate with folic acid was reported not to increase the CSF folate levels, while the levels increased when 5-formyltetrahydrofolate (Leucovorin) was used (Mattson, Gallagher, Reynolds and Glass, 1973). The reason postulated was that the anticonvulsant could be interfering with the conversion of folic acid to the active reduced form (5-methyltetrahydrofolate), a form which enters the CSF, hence preventing its entry to the CSF (Levitt, Nixon, Pincus and Bertino, 1971). In addition, phenytoin and phenobarbitone serum and CSF concentrations were reported to be reduced during folic acid treatment, especially phenobarbitone (Mattson et al., 1973). Cerebrospinal fluid amine metabolites, i.e. 5-hydroxy-3-indole acetic acid (HIAA) and possibly homovanilic acid (HVA), were reported to have higher concentrations in epileptic patients on anticonvulsant therapy, especially for HIAA in patients with high levels of serum anticonvulsants (Chadwick, Jenner and Reynolds, 1975), and it was thought that/...



that reduced CSF folate might have some correlation with the increased levels of the amine metabolites (Reynolds, Chadwick, Jenner and Chanarin, 1975). This possibility of correlating folate with catecholamine and indolamine metabolism has been put forward because of reports on the existence in several species of a brain methyltransferase requiring 5-methyltetrahydrofolate as a methyl donor and capable of N-methylation of nearly all primary or secondary catecholamines or indoleamines in vitro (Lauduron, 1972; Leyson and Lauduron, 1974).

In this study, the CSF amine metabolites were measured before and after treatment with either folic acid or 5-formyltetrahydrofolate as they have been reported to be increased especially for HIAA in drug treated epileptic patients (Reynolds et al., 1975), and it was interesting to find whether treatment with either folic acid or 5-formyltetrahydrofolate would affect their levels and whether or not this has any relevance to their epilepsy control.

#### METHODS

Seventeen patients were studied. They were eight females and nine males, age range 20-71 years (mean 40 years). They were on multiple anticonvulsant drug therapy, including phenytoin, phenobarbitone, primidone in/...

in various combinations, in addition to carbamazepine, sodium valproate, clonazepam and ethotoin. All the patients had been on anticonvulsants for at least 10 years.

Cerebrospinal fluid was obtained, within one hour of awakening in the morning, by lumbar puncture and blood samples were taken at the same time.

In the CSF, folate was measured in all the patients by the *Lactobacillus Casei* method, but successful HVA and HIAA measurements were done in only seven patients before and after treatment with either folic acid or 5-formyl-tetrahydrofolate. Cerebrospinal fluid HVA and HIAA were measured by fluorimetry.

The patients were divided into two groups. The first group, consisting of nine patients, were started on 3 mg of 5-formyltetrahydrofolate intramuscularly daily for four weeks; in only four of them the CSF HVA and HIAA were estimated before and after the end of the treatment period. The second group, consisting of eight patients, were started on folic acid 5 mg t.i.d. for four weeks; in this group CSF HVA and HIAA were measured in only three patients before and after the treatment period.

Serum anticonvulsants, including phenytoin, phenobarbitone and primidone, were measured by gas chromatography in all patients before and after the end of the treatment/...

treatment periods, and the dose of the anticonvulsants was kept constant for three months before and three months after the four week treatment period. The measurements were repeated three months after the end of the treatment period.

For details of the techniques used see under METHODS.

Seizure frequency was recorded as major and minor seizures (see Chapter 1).

## RESULTS

### Serum folate

The results of the serum folate levels for the nine patients in the group which started on 5-formyltetrahydrofolate had a mean of  $1.58 \mu\text{g/l} \pm 0.42$  S.D. The levels increased after four weeks of treatment to a mean of  $10.7 \mu\text{g/l} \pm 3.65$  S.D. In the second group, which started on folic acid treatment, the serum folate in eight patients, before the treatment started, had a mean of  $1.28 \mu\text{g/l} \pm 0.64$  S.D., and it increased to  $11.36 \mu\text{g/l} \pm 3.5$  S.D. There was no significant difference between the post-treatment folate levels in the two groups.

### Cerebrospinal fluid folate levels

In the first group, i.e. the nine patients who had

5/...

5-formyltetrahydrofolate, the CSF folate increased from 8.88  $\mu\text{g}/\text{l} \pm 4$  S.D. to 41.66  $\mu\text{g}/\text{l} \pm 7.3$  S.D. In the group who had folic acid treatment, the CSF folate similarly increased from a mean of 8.27  $\mu\text{g}/\text{l} \pm 3.8$  S.D. to 31.6  $\mu\text{g}/\text{l} \pm 10.7$  S.D. However, the post-treatment CSF folate levels showed a significant difference. The group who were on 5-formyltetrahydrofolate had the highest increase ( $t = 1.86$ ,  $P < 0.025$ , using Student's  $t$  test).

The serum and CSF folate did not show a significant correlation in all of the patients before treatment. After treatment in the group who received 5-formyltetrahydrofolate, there was a significant correlation ( $y = 1.69x + 23.46$ ;  $P < 0.01$ ) (Fig. 7.1). However, in the group who had folic acid treatment, the correlation between serum and CSF folate levels remained insignificant.

#### Seizure frequency

The number of seizures/patient/month for the three months before treatment with either substance, during the month of actual therapy and for three months after the end of therapy did not show any significant difference in either group using Wilcoxon signed-ranks test (Fig. 7.2).

#### Cerebrospinal fluid HVA and HIAA

Both HVA and HIAA were measured successfully before and after treatment in both groups in only seven patients and/...

Fig. 7.1 Serum and CSF folate in  $\mu\text{g}/\text{l}$ , in nine previously folate deficient patients. The patients had four weeks treatment with 5-formyltetrahydrofolate.  $y = 1.69x + 23.46$  ( $P < 0.01$ ).

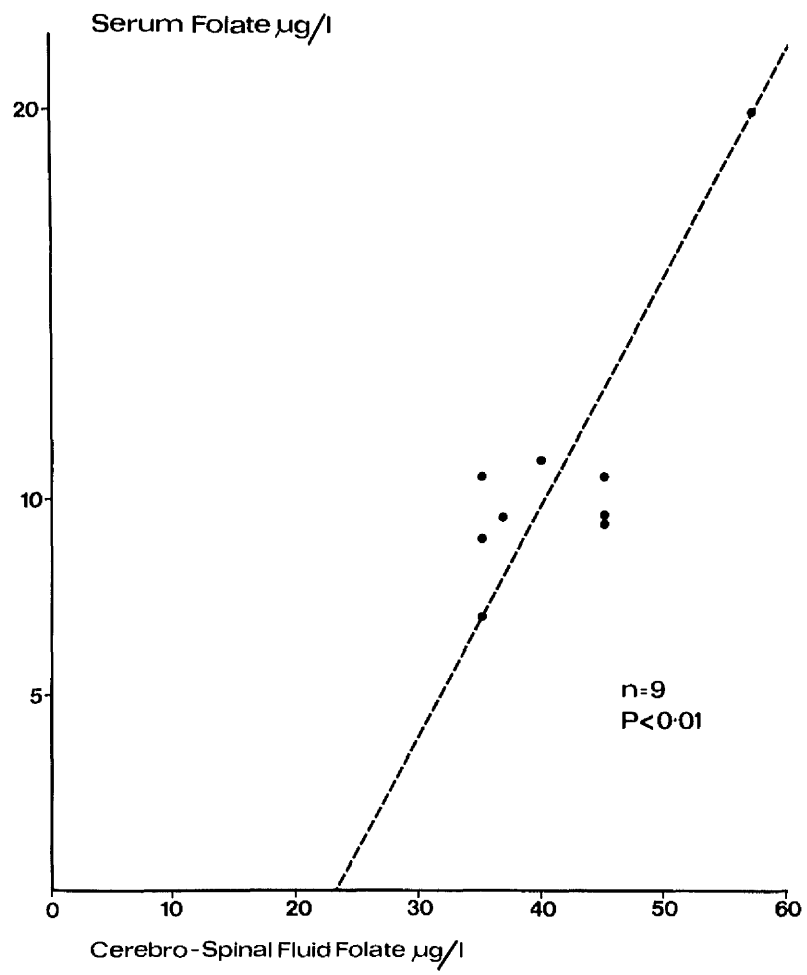


Fig. 7.2 Number of seizures/patient/month. During the three months before starting treatment, in the treatment month, and for three months after the end of the treatment. Both groups showed no significant change, using Wilcoxon signed-ranks test.

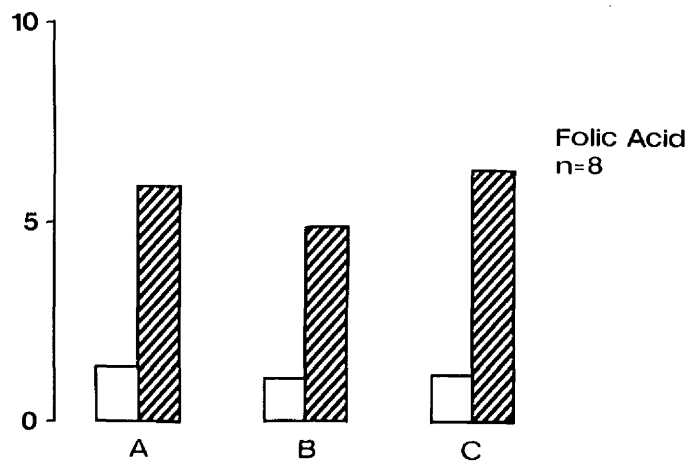
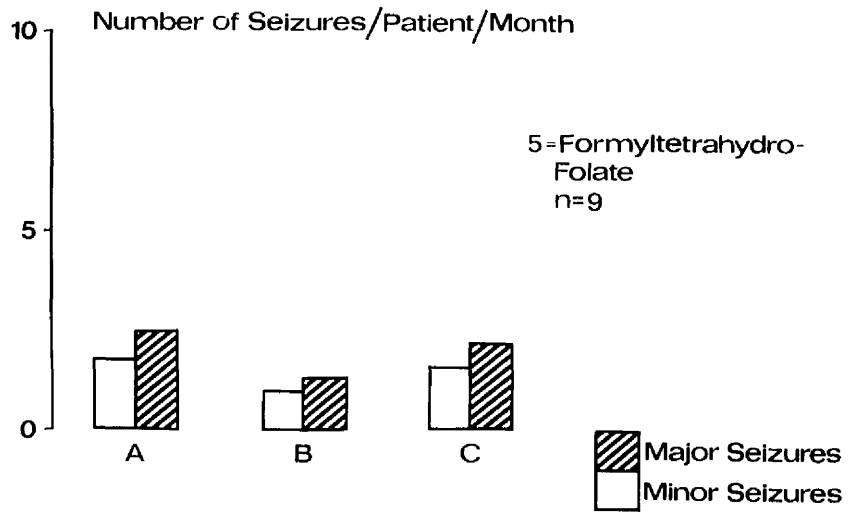
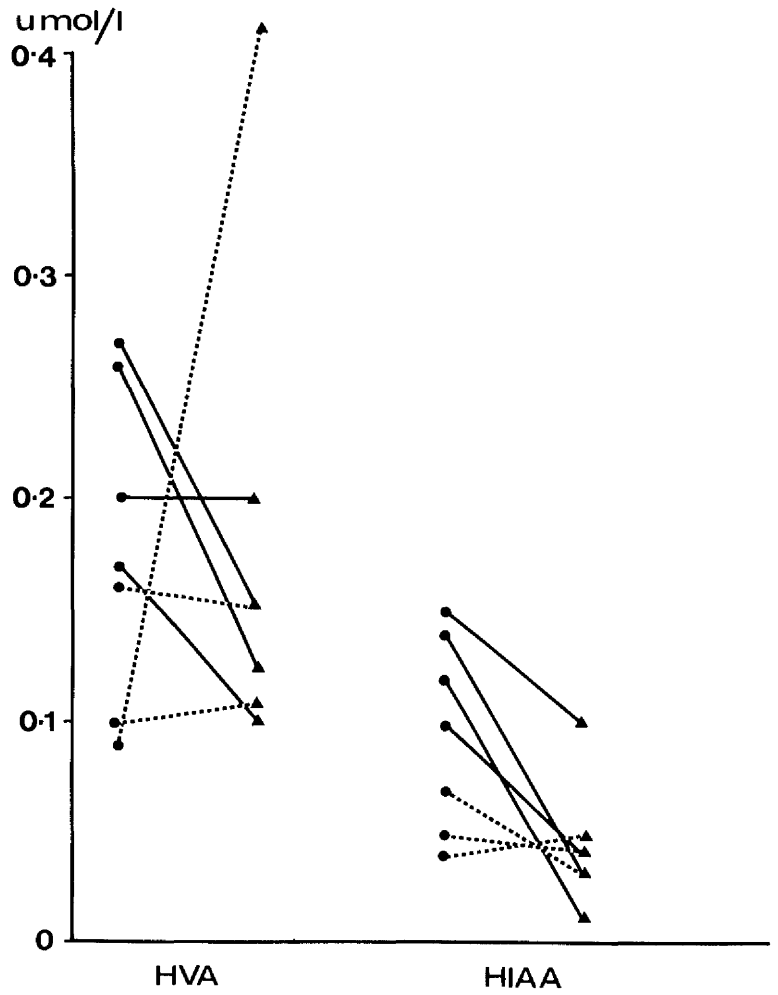
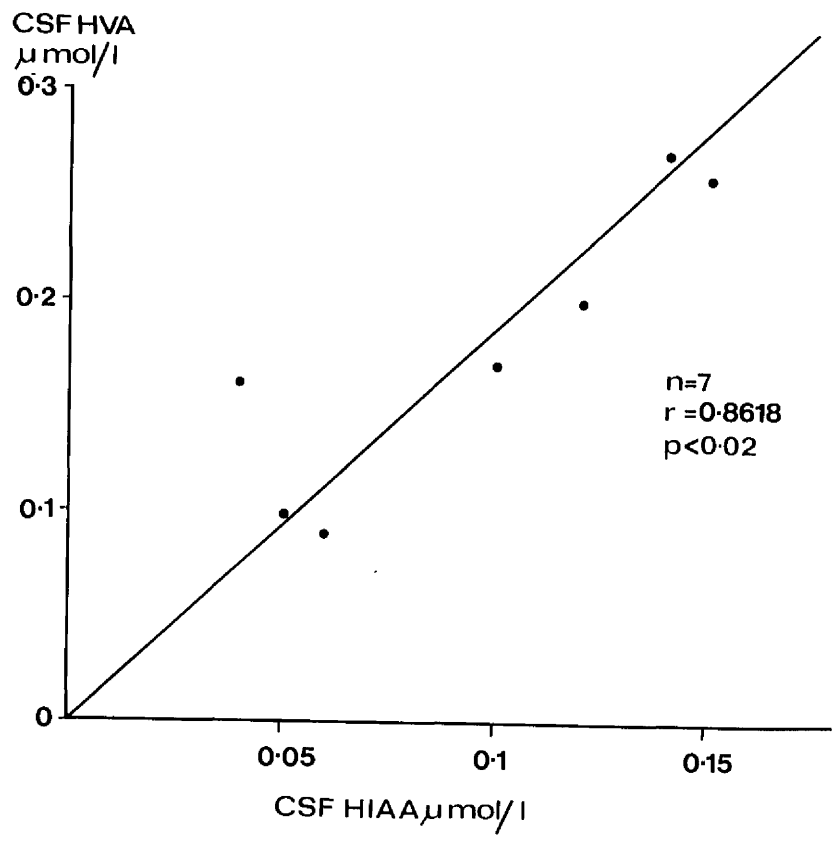




Fig. 7.3 Correlation between CSF HVA and HIAA in seven folate deficient epileptic patients before start of therapy.  $y = 0.0009 + 0.53x$ ,  $t = 0.86$  ( $P < 0.02$ ).

Fig. 7.4 Cerebrospinal fluid HIAA and HVA in patients on 5-formyltetrahydrofolate (—), and folic acid (---). Values before (⊙) and after (▲) treatment.



and the main difficulty was obtaining enough CSF for the assay. Four of the patients were in the group which received 5-formyltetrahydrofolate and three were in the group which was treated with folic acid. There was no difference between the two groups prior to treatment and the correlation between HIAA and HVA is shown in Fig. 7.3. After treatment, both HVA and HIAA were reduced in all four patients who were on 5-formyltetrahydrofolate, but those who were on folic acid did not show a consistent change. The CSF HIAA in the four patients before treatment with 5-formyltetrahydrofolate was  $0.127 \mu\text{mol/l} \pm 0.02 \text{ S.D.}$ , and it was reduced after treatment to  $0.045 \mu\text{mol/l} \pm 0.036 \text{ S.D.}$  ( $P < 0.01$ , using Student's t test). CSF HVA before treatment was  $0.225 \mu\text{mol/l} \pm 0.047 \text{ S.D.}$ , and it was reduced to  $0.142 \mu\text{mol/l} \pm 0.042 \text{ S.D.}$  ( $P < 0.05$ , using Student's t test). All results for both groups are shown in Fig. 7.4.

#### Serum anticonvulsant levels

Table 7.1 shows the results, mean  $\pm$  S.D., of serum phenytoin, phenobarbitone and primidone concentrations in both groups of patients. The only significant change was in patients who received 5-formyltetrahydrofolate; there was a significant reduction in serum phenytoin level immediately after the end of the four week treatment period ( $P < 0.05$ , using Student's t test). The serum phenytoin was similarly/...

Serum Anticonvulsants ( $\mu\text{mol/l}$ )

Patients on 5-formyltetrahydrofolate			
	phenytoin(7)	phenobarbitone(9)	primidone(4)
Before treatment	47.2 $\pm$ 24 SD	113.4 $\pm$ 60.5 SD	24.7 $\pm$ 17 SD
After treatment	29.6 $\pm$ 11.6 SD	141.5 $\pm$ 99.1 SD	29.9 $\pm$ 15.1 SD
Three months later	52.4 $\pm$ 46 SD	161.7 $\pm$ 107.5 SD	27.9 $\pm$ 19.7 SD
Patients on folic acid			
	phenytoin(8)	phenobarbitone(8)	primidone(6)
Before treatment	48.4 $\pm$ 22.4 SD	110 $\pm$ 53.3 SD	29.9 $\pm$ 16.1 SD
After treatment	40 $\pm$ 17.6 SD	145.3 $\pm$ 64.2 SD	32.2 $\pm$ 19.7 SD
Three months later	49.2 $\pm$ 31.6 SD	172.2 $\pm$ 102.4 SD	26.6 $\pm$ 4.4 SD

Table 7.1 : Serum anticonvulsants before, immediately after, and three months after the end of treatment. Serum phenytoin was significantly reduced immediately after the end of treatment with 5-formyltetrahydrofolate ( $P < 0.05$ , using Student's  $t$  test).

similarly reduced in the group that received folic acid, but this was not statistically significant. There was a trend for the serum phenobarbitone levels both in patients who were on the drug itself and those who were on primidone to increase whether they were treated with 5-formyltetrahydrofolate or with folic acid. However, this was not statistically significant.

#### DISCUSSION

The effects of both folic acid and 5-formyltetrahydrofolate on the serum folate levels did not show any difference, as was expected. One of the main points behind this work was to see how much treatment with folic acid or 5-formyltetrahydrofolate would affect the CSF folate concentration. Treatment of folate deficient epileptic patients with folic acid has been reported not to increase the CSF folate concentration to the normal levels. However, this was readily achieved when the reduced form, 5-formyltetrahydrofolate, was used, and it was presumed that the anticonvulsant drug interfered with conversion of folic acid to 5-methyltetrahydrofolate (Mattson et al., 1973). This supports the findings in this study in that the CSF folate, after four weeks of treatment with both folic acid and 5-formyltetrahydrofolate, increased, but the increase with the latter was more, and the/...

the difference between the two was statistically significant. This may indicate that there is indeed an effect by the anticonvulsant on the conversion of folic acid to 5-methyltetrahydrofolate. The CSF levels of intravenously injected radioactive folic acid, 5-formyltetrahydrofolate and 5-methyltetrahydrofolate have been reported to show that only 5-methyltetrahydrofolate was transported through to the CSF (Levitt et al., 1971). These authors also showed that administration of phenytoin did not interfere with this mechanism. In this study, 5-formyltetrahydrofolate was used and it has previously been shown to be readily converted to 5-methyltetrahydrofolate (Nixon and Bertino, 1972). The serum and CSF folate before starting treatment did not show a significant correlation, most likely because the patients chosen for CSF folate estimation were those who had the lowest serum folate levels (Chapter 6). After treatment, the group who received 5-formyltetrahydrofolate showed a significant correlation, indicating a return to the normal distribution of folate in both blood and CSF (Reynolds, Gallagher, Mattson, Bowers and Johnson, 1972). However, the group who received folic acid failed to show this return to normal correlation, therefore indicating an interference in the conversion of folic acid to 5-methyltetrahydrofolate.

There have been several reports on the effects of folate/...

folate treatment on the seizure control in folate deficient epileptic patients. Worsening of seizure control has been reported (Reynolds and Wales, 1967). However, later reports did not substantiate this (Jensen and Olesen, 1970; Norris and Pratt, 1971), and the latter finding is consistent with results in this study. Folic acid treatment was noted to reduce serum phenytoin concentration (Jensen and Olesen, 1970; Mattson et al., 1973). In this study, there was a significant reduction in the serum phenytoin concentration in those patients who received 5-formyltetrahydrofolate although there was a reduction with folic acid treatment, but this has failed to reach significance. The explanation of this is not yet clear and the previous suggestion of an increase in para-hydroxylation of phenytoin still needs to be investigated (Kutt, Winter and McDowell, 1966). The reduction in the serum phenytoin concentration being more significant when the reduced form of folate was used, supports the idea that phenytoin prevents the conversion of folic acid to 5-methyltetrahydrofolate and thereby the reduction in serum phenytoin concentration is less pronounced when folic acid is used rather than 5-formyltetrahydrofolate.

Folic acid when given by mouth has been shown to reduce cerebrospinal fluid HVA and to a less extent HIAA. This/...

This effect was less marked in patients on anticonvulsants (Hunter, Barnes, Curzon, Kantamaneni and Duncan, 1971). CSF amine metabolites have been reported to be raised in epileptic patients when compared to untreated epileptics and controls (Chadwick, Jenner and Reynolds, 1975). Cerebral amine metabolism has been linked, especially that of 5-hydroxytryptophan, to anticonvulsant drug effect (Meyer and Frey, 1973). However, the results shown in this study point to the reduction of CSF HIAA and HVA after increasing the serum folate with 5-formyltetrahydrofolate. This is perhaps very important because it may indicate that the CSF amine metabolites are not related to the serum anticonvulsant level directly as has been suggested (Chadwick et al., 1975), but indirectly through folate depletion, and the idea that there is an exit block to the amine metabolites may be true, on the assumption that folate acts as a methyl donor in indoleamine metabolism (Korevaar, Geyer, Knap, Hsu and Mandell, 1973). This hypothesis of blocked exit has previously been suggested (Chadwick et al., 1975) and it has been shown that intraventricularly injected 5HIAA is retained for a longer time in rats receiving anticonvulsants (Chase, Katz and Kopin, 1969).

The reduction of CSF HIAA and HVA with treatment on the one hand and the maintenance of the same seizure frequency/...



frequency on the other may lead to the conclusion that the dependence of anticonvulsant drug action on central amines, which has been reported previously in mice, has a less effective role in humans (Meyer and Frey, 1973).

It seems possible that the relation of anticonvulsant effect on central amine metabolism is through its effects on folate. Although the number of patients studied was small, the results were in agreement with other workers. However, the new finding of reduction of CSF amines and its implication as regards the role of folate in catecholamine and indoleamine metabolism need further confirmation.

## SUMMARY

1. Seventeen folate deficient epileptic patients were divided into two groups, and treated with either folic acid or its reduced form, 5-formyltetrahydrofolate, for a period of four weeks.
2. Serum folate increased after treatment with both substances, but the CSF folate increased more significantly when the reduced form was used, indicating that the anticonvulsant may hinder the conversion of folic acid to 5-methyltetrahydrofolate.
3. Serum phenytoin levels were significantly reduced after treatment with 5-formyltetrahydrofolate.
4. Cerebrospinal fluid HIAA and HVA levels were reduced after treatment with 5-formyltetrahydrofolate, which may lead to the conclusion that there is an exit block in the central amine metabolism, secondary to folate depletion.
5. There was no effect on the seizure frequency in all the patients examined.

## SUGGESTIONS FOR FURTHER STUDY

It is evident from the summary of this thesis that there is a long way to go until an ideal anticonvulsant is available. However, any new drug which is added to the list of anticonvulsant agents needs close and continuous examination. I have tried to do this in the first section of this thesis, although in a limited way, i.e. by adding either drug to other medication. This may give some information as regards the interaction between drugs. It may also give information on their effectiveness as long as other parameters are kept constant. In this context, a study is needed where newly diagnosed epileptic patients receive only the trial drug, i.e. clonazepam or sodium valproate, as a sole anticonvulsant, thereby eliminating all possibilities of drug interactions, and the effects and side effects would be observed over a period of time.

The effect of sodium valproate on phenobarbitone metabolism needs further study. By giving patients, who are on phenobarbitone only, a large dose of sodium valproate, and studying the serum and urinary levels of phenobarbitone, one could try to establish where sodium valproate acts, whether it is in the absorption, hepatic metabolism, or in the excretion of the drug.

The relation between reduced serum IgA levels during/...

during phenytoin therapy and the HLA system needs confirmation. A larger number of patients is needed. However, it seems a fascinating idea that the immune system is affected by drugs in only susceptible individuals who possess certain genetic markers. The implications of this as regards predisposition to disease may be explored further.

The role of brain amine metabolites during drug treatment of epilepsy does not seem to be fully investigated yet. The number of patients in this thesis is small, but there was no striking change in their seizure frequency. This may perhaps lead to the conclusion that brain amines, which were reduced when 5-formyltetrahydrofolate was given, do not play a major role in the anticonvulsant action of the anti-epileptic agents. This needs further investigation by treating a larger number of patients and observing any change in their seizure frequency after reducing their brain amines.

Finally, I feel strongly that the search for the ideal anticonvulsant should be one of the main targets of research. I hope that man will be the victor in the end.

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## PUBLICATIONS AND COMMUNICATIONS RESULTING FROM THIS WORK

1. Comparison of sodium valproate (Epilim) and clonazepam (Rivotril) in intractable epilepsy (preliminary report) (1976). Shakir, R.A., Johnson, R.H., Keogh, H.J., Lambie, D.G. & Nanda, R.N. In Epilepsy. The Eighth International Symposium. Ed. J.K. Penry, Raven Press: New York, pp. 181-186.
  
2. Metabolism of immunoglobulin A, lymphocyte function and histocompatibility antigens in patients on anticonvulsants. Shakir, R.A., Behan, P.O., Dick, N. & Lambie, D.G. J. Neurol. Neurosurg. Psychiat. (in press).
  
3. Comparative trial of sodium valproate and clonazepam in chronic epilepsy. Shakir, R.A., Johnson, R.H., Lambie, D.G. & Nanda, R.N. Arch. Neurol. (in press).