membrane-bound enzyme activities may change as a consequence and that the cation transport mechanisms of the resulting ghosts may be different from those of the original cells (for a review see Schwoch & Passow, 1973). It may be that such changes during the preparation of erythrocyte ghosts render the Na^+, K^+ -ATPase more susceptible to the effects of phenytoin *in vitro* resulting in the effects observed by Scott *et al.* (1980).

We gratefully acknowledge the co-operation of our colleagues in the Department of Neurology in the Radcliffe Infirmary for allowing us to study patients in their care. The plasma anticonvulsant concentra-

tions were measured by the routine service of the Department of Clinical Biochemistry in the John Radcliffe Hospital, Oxford. JKA is supported by the Wellcome Trust.

J.K. ARONSON, D.P. GEANEY, D.G. GRAHAME-SMITH, J.C. CARVER & K.F. HALLIS

MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE

Received February 19, 1981

References

- ARONSON, J.K., GRAHAME-SMITH, D.G., HALLIS, K.F., HIBBLE, A. & WIGLEY, F. (1977). Monitoring digoxin therapy: I. Plasma concentrations and an *in vitro* assay of tissue response. *Br. J. clin. Pharmac.*, **4**, 213–221.
- CUMBERBATCH, M., ZAREIAN, K., DAVIDSON, C., MORGAN, D.B. & SWAMINATHAN, R. (1981). The early and late effects of digoxin treatment on the sodium transport, sodium content and Na^+, K^+ -ATPase of erythrocytes. *Br. J. clin. Pharmac.* (in press).
- DEUPREE, J.D. (1977). The role or non-role of ATPase activation by phenytoin in the stabilization of excitable membranes. *Epilepsia*, **18**, 309–315.
- FORD, A.R., ARONSON, J.K., GRAHAME-SMITH, D.G., ROSE, J.A. (1979a). The characteristics of the binding of 12- α -[^3H]-digoxin to the membranes of intact human erythrocytes: relevance to digoxin therapy. *Br. J. clin. Pharmac.*, **8**, 115–124.
- FORD, A.R., ARONSON, J.K., GRAHAME-SMITH, D.G. & CARVER, J.G. (1979b). Changes in cardiac glycoside receptor sites $^{86}\text{rubidium}$ uptake and intracellular sodium concentrations in the erythrocytes of patients receiving digoxin during the early phases of treatment of cardiac failure in regular rhythm and of atrial fibrillation. *Br. J. clin. Pharmac.*, **8**, 125–134.
- KURATA, D. & WILKINSON, G.R. (1974). Erythrocyte uptake and plasma binding of diphenylhydantoin. *Clin. Pharmac. Ther.*, **16**, 355–362.
- SCHWOCH, G. & PASSOW, H. (1973). Preparation and properties of human erythrocyte ghosts. *Mol. Cell. Biochem.*, **2**, 197–218.
- SCOTT, A.K., GILBERT, J.C. & WEBSTER, J. (1980). Anticonvulsants and human erythrocyte ATPase activities. *Br. J. clin. Pharmac.*, **10**, 175–177.

VALPROIC ACID FAILS TO DISPLACE PHENOBARBITONE AND CARBAMAZEPINE FROM PLASMA PROTEIN BINDING SITES IN EPILEPTIC PATIENTS

Valproic acid is extensively (90% approximately) bound to plasma proteins (Gugler & Mueller, 1978) and therefore the possibility that it may displace other drugs from binding sites needs to be considered. In a recent study (Pisani & Di Perri, 1981), we have shown that a single dose of sodium valproate given intravenously to epileptic patients stabilized on phenytoin therapy results in a significant increase in the saliva: plasma ratio of the latter drug. Since this reflects the unbound fraction in plasma (Troupin & Friel, 1975; McAuliff *et al.*, 1977), these data indicate that valproic acid displaces phenytoin from plasma proteins in agreement with previous reports (Mattson *et al.*, 1978; Monks & Richens, 1980; Perucca *et al.*, 1980). Whether valproic acid could also affect the plasma protein binding of other antiepileptic drugs

has been little investigated. Jordan, Shillingford & Steed (1976) observed *in vitro* a significant displacement of phenobarbitone by valproic acid. In order to examine this possibility we have studied the effect of a single dose of sodium valproate on the steady-state concentration of phenobarbitone and carbamazepine in the plasma and saliva of patients with epilepsy.

Ten patients (six males, four females) aged 14–37 years and weighing 49–78 kg, were studied. Five patients were treated with phenobarbitone alone (100–250 mg/day) and five with carbamazepine alone (1000–1200 mg/day). Plasma and mixed saliva samples were collected for each patient immediately before and 15 and 45 min after intravenous administration of single dose (1200 mg) of sodium valproate (Sigma-Tau, Italy). The plasma and saliva levels of

Table 1 Plasma and saliva levels of PB and CBZ and their ratios in epileptic patients at different time intervals after i.v. administration of a single dose of 1200 mg sodium valproate

Time (min)	PBp ($\mu\text{g/ml}$)	PBs ($\mu\text{g/ml}$)	PBs/p (%)	CBZp ($\mu\text{g/ml}$)	CBZs ($\mu\text{g/ml}$)	CBZs/p (%)
0	28.4 $\pm$ 2.4	8.6 $\pm$ 0.6	30.4 $\pm$ 1.2	9.6 $\pm$ 0.3	3.4 $\pm$ 0.2	36.1 $\pm$ 1.6
15	28.6 $\pm$ 2.3	8.4 $\pm$ 0.8	29.3 $\pm$ 1.1	9.7 $\pm$ 1.0	3.5 $\pm$ 0.3	36.2 $\pm$ 1.0
45	27.6 $\pm$ 2.0	8.3 $\pm$ 0.8	30.1 $\pm$ 1.0	9.1 $\pm$ 0.6	3.4 $\pm$ 0.2	37.5 $\pm$ 1.5

PB = phenobarbitone; CBZ = carbamazepine; p = plasma; s = saliva. Values are expressed as means $\pm$ s.e. mean.

phenobarbitone and carbamazepine were determined in triplicate by enzyme immunoassay (EMIT®), this technique showing reliable accuracy and reproducibility also for saliva assay of antiepileptic drugs (Schottelius, 1978; Monaco *et al.*, 1979; Paxton & Donald, 1980). Valproic acid in plasma was determined in duplicate by gas-liquid chromatography (Kupferberg, 1978). Statistical analysis was performed by means of Student's *t*-test for paired data.

Results of the study are summarized in Table 1. The saliva: plasma ratios of phenobarbitone and carbamazepine at time 0 were 30.4 $\pm$ 1.2 and 36.1 $\pm$ 1.6 (mean $\pm$ s.e. mean), respectively (Table 1). After administration of sodium valproate, the levels of the drugs in plasma and in saliva, and their saliva: plasma ratios showed no significant change throughout the period of observation (Table 1). Plasma valproic acid levels determined at 15 and 45 min were 107 $\pm$ 2.3 and 89.8 $\pm$ 2.5 (mean $\pm$ s.e. mean) $\mu\text{g/ml}$, respectively (Table 1).

It has previously been shown that the saliva: plasma ratios of phenobarbitone and carbamazepine are positively correlated with the free fraction in plasma (Troupin & Friel, 1975; McAuliffe *et al.*, 1977). Our values of saliva: plasma ratios of carbamazepine were higher than those obtained by other authors (Johannessen *et al.*, 1976; Westenberg *et al.*, 1978; Paxton & Donald, 1980) but this discrepancy may have been due to cross-reactivity of carbamazepine-epoxide with carbamazepine, when using EMIT® for measurement in saliva (Monaco & Piredda, 1980).

The fact that, after valproate administration, the saliva:plasma ratios of phenobarbitone and carbamazepine remained unchanged suggests that the plasma protein binding of both drugs was not appreciably affected by valproic acid. Displacement of drug from plasma proteins would be expected to result in (1) a fall in total (i.e. free + bound) plasma levels, (2) an increase in the free fraction, and (3) an unmodified level of the free drug in plasma. In a recent study, for instance, Paxton (1980) found that aspirin is able to displace phenytoin from plasma proteins without modifying salivary levels. These results are in good agreement with other studies in

which the free phenytoin levels remained unchanged in the presence of displacing agents such as salicylate (Fraser *et al.*, 1980) and valproic acid (Mattson *et al.*, 1978; Monks & Richens, 1980), but conflict with the data of other authors who observed an increase in the saliva phenytoin levels following sodium valproate (Perucca *et al.*, 1980; Pisani & Di Perri, 1981) and salicylate (Leonard *et al.*, 1981). Probably factors other than displacement from plasma proteins, such as inhibition of metabolism or displacement from tissues, may be involved. In all of these studies, the presence of the displacing agent resulted in a fall in total (i.e. free + bound) phenytoin plasma levels. This is to be expected because phenytoin, like phenobarbitone and carbamazepine, has a relatively large apparent volume of distribution and a displacement from plasma proteins should result in a rapid transfer of the free drug to extravascular compartments with a consequent fall in total drug level (Perucca & Richens, 1980). Although this effect is expected to be of a lesser magnitude for phenobarbitone and carbamazepine, since these drugs have a lower degree of plasma protein binding, no significant change was observed in the present study. We conclude that valproic acid at the concentrations obtained does not affect the plasma protein binding of phenobarbitone and carbamazepine in epileptic patients.

We are very grateful to Professor A. Richens and to Dr Emilio Perucca for their frequent advice during the preparation of the manuscript.

We would also like to thank the Italian Sigma-Tau Company for supplying the sodium valproate vials.

FRANCESCO PISANI,* GIANCARLA OTERI & RAOUL DI PERRI
Clinica Neurologica dell 'Università', Policlinico
Gazzi, 98013 Contesse-Messina, Italy

Received February 23, 1981

* Present address: Department of Pharmacology, Welsh National School of Medicine, Heath Park, Cardiff, United Kingdom.

References

- FRASER, D.G., LUOLDEN, T.M., EVANS, R.P. & SUTHERLAND, E.W. (1980). Displacement of phenytoin from plasma binding sites by salicylate. *Clin. Pharmac. Ther.*, **27**, 165–169.
- GUGLER, R. & MUELLER, G. (1978). Plasma protein binding of valproic acid in healthy subjects and in patients with renal disease. *Br. J. clin. Pharmac.*, **5**, 441–446.
- JOHANNESSEN, S.I., GERNA, M., BAKKE, J., STANDJORD, R.E. & MORSELLI, P.L. (1976). CSF concentration and serum protein binding of carbamazepine and carbamazepine-10-11 epoxide in epileptic patients. *Br. J. clin. Pharmac.*, **3**, 575–582.
- JORDAN, B., SHILLINGFORD, J.S. & STEED, K.P. (1976). Preliminary observations on protein-binding and enzyme-inducing properties of sodium valproate (Epilim). In *Clinical and Pharmacological Aspects of Sodium Valproate (Epilim) in the Treatment of Epilepsy*, ed. Legg, N.J., pp. 112–116. Tunbridge Wells: MCS Consultants.
- KUPFERBERG, H. (1978). Gas-liquid Chromatographic Quantitation of Valproic Acid. In *Antiepileptic Drugs: Quantitative Analysis and Interpretation*, eds. Pippenger, C.E., Penry, J.K. & Kutt, H., pp. 147–151. New York: Raven Press.
- LEONARD, R.F., KNOTT, P.J., RANKIN, G.O., ROBINSON, D.S. & MELNICK, D.E. (1981). Phenytoin-salicylate interaction. *Clin. Pharmac. Ther.*, **29**, 56–60.
- MATTSON, R.H., CRAMER, J.A., WILLIAMSON, P.C. & NOVELLY, R.A. (1978). Valproic acid in epilepsy: clinical and pharmacological effects. *Ann. Neurol.*, **3**, 20–25.
- McAULIFFE, J.J., SHERWIN, A.L., LEPIK, I.E., FAYLE, S.A. & PIPPENGER, C.E. (1977). Salivary levels of anticonvulsants: a practical approach to drug monitoring. *Neurology*, **27**, 409–413.
- MONACO, F., MUTANI, R., MASTROPAOLO, C. & TONDI, M. (1979). Tears as the best practical indicator of the unbound fraction of an anticonvulsant drug. *Epilepsia*, **20**, 705–710.
- MONACO, F. & PIREDDA, S. (1980). Carbamazepine-10,11-epoxide determined by EMIT carbamazepine reagent. *Epilepsia*, **21**, 475–477.
- MONKS, A. & RICHENS, A. (1980). Effect of single doses of sodium valproate on serum phenytoin levels and protein binding in epileptic patients. *Clin. Pharmac. Ther.*, **27**, 89–95.
- PAXTON, J.W. (1980). Effects of aspirin on salivary and serum phenytoin kinetics in healthy subjects. *Clin. Pharmac. Ther.*, **27**, 170–178.
- PERUCCA, E., HEBDIGE, S., GATTI, G., LECCHINI, S., FRIGO, G.M. & CREMA, A. (1980). Interaction between phenytoin and valproic acid: plasma protein binding and metabolic effects. *Clin. Pharmac. Ther.*, **28**, 779–789.
- PERUCCA, E. & RICHENS, A. (1980). Interpretation of drug levels: relevance of plasma protein binding. In *Drug Concentration in Neuropsychiatry*, pp. 52–68, Amsterdam: Excerpta Medica.
- PISANI, F. & DI PERRI, R. (1981). Intravenous valproate: effects on plasma and saliva phenytoin levels. *Neurology*, **21**, 467–470.
- SCHOTTELIUS, D.O. (1978). Homogeneous immunoassay system (EMIT) for quantitation of antiepileptic drugs in biological fluids. In *Antiepileptic Drugs: Quantitative Analysis and Interpretation*, eds. Pippenger, C.E., Penry, J.K. & Kutt, H., pp. 95–108. New York: Raven Press.
- TROUPIN, A.S. & FRIEL, P. (1975). Anticonvulsant level in saliva, serum and cerebrospinal fluid. *Epilepsia*, **16**, 223–227.
- WESTENBERG, H.G.M., VAN DER KLEIJN, E., OEI, T.T. & DE ZEEUW, R.A. (1978). Kinetics of carbamazepine and carbamazepine-epoxide, determined by use of plasma and saliva. *Clin. Pharmac. Ther.*, **23**, 320–328.

EFFECTS OF CONCURRENT ADMINISTRATION OF OTHER SUBSTRATES OF N-ACETYLTRANSFERASE ON DAPSONE ACETYLATION

Acetylation is a principal route for metabolism of arylamines. *N*⁴-acetylation of certain sulphonamides is often utilised to delineate the capacity of the *N*-acetyltransferase system to metabolise other drugs, e.g. dapsone (DDS), hydralazine (HYD), isoniazid (INH), procainamide and phenelzine. Individuals may be classified by acetylation rate, this characteristic being bimodally distributed with slow acetylation as an autosomal Mendelian recessive and rapid acetylation a dominant trait. Sulphamethazine (SMZ) is widely used to distinguish slow and fast acetylators by determination of the ratio of the *N*-acetylsulphamethazine (NSMZ) to SMZ in the plasma and urine (Evans, 1969). A similar division can be made on the basis of the ratio of monoacetyl-

dapsone (MADDS) to DDS in the plasma (Gelber *et al.* 1971). Several experiments with different substrates have suggested that the human *N*-acetyltransferase system is saturable (Graffner, 1975; Ellard & Gammon, 1976; Talseth, 1977; Olson, Miceli & Weber, 1978; du Souich *et al.*, 1979). Competition between substrates might therefore occur and provide further evidence of saturability within the clinical dosage range.

Normal, healthy volunteers gave their informed consent to take the following drugs orally in the fasted state: DDS (50 mg); SMZ (10 mg/kg); DDS (50 mg) and SMZ (10 mg/kg); DDS (50 mg) and INH (100 mg); SMZ (10 mg/kg) and HYD (50 mg).

Twenty-one out-patients attending the London