Interference by Certain Anticonvulsant Drugs with Assay of Barbiturate in Plasma

To the Editor:

While estimating barbiturates in the plasma of patients who were taking several anticonvulsant drugs simultaneously, we observed distortion of the barbiturate ultraviolet absorption curves when Broughton's spectrophotometric method (1) was used (Figures 1 and 2).

We therefore systematically measured the interference of commonly-used anticonvulsants with the Broughton (1) and Jatlow (2) spectrophotometric methods by adding these drugs, in a series of concentrations or near their therapeutic ranges in plasma, to aqueous solutions containing a known quantity of sodium diethylbarbiturate (25 mg/liter). We also added other anticonvulsants in a similar range of concentrations to the plasma of patients who were taking phenobarbital only, and whose plasma was known to contain therapeutic quantities of the drug. We compared the data thus obtained with the results of gas-liquid chromatographic (GLC) examination of similar samples, using our modification (3) of Toseland's method (4), which is in routine use in our laboratory for the estimation of phenobarbital, phenytoin, and primidone.

We found that pheneturide [phenyl-ethylyacetetylurea] in concentrations exceeding 10 mg/liter and ethosuximide in concentrations exceeding 60 mg/liter caused significant under-estimation of barbiturate in aqueous solution or in plasma when Broughton's scan was used, and to a lesser extent in plasma when Jatlow's scan was used. When ethosuximide at the high therapeutic concentration of 80 mg/liter was added to plasma already containing 18 mg of phenobarbital per liter, the concentration of this drug was underestimated by 10% when Jatlow's scan was used, but by 30% when the Broughton scan was used, while addition of pheneturide (10 mg/liter) to plasma already containing 15 mg of phenobarbital per liter caused underestimation of 22% with Jatlow's scan and 67% with Broughton's scan.

Sulthiane [BPC name; Sulthiane, WHO name] gave rise to the greatest interference, producing gross distortion of the ultraviolet scan (Figure 2). In concentrations exceeding 5 mg/liter this drug caused underestimation of barbiturate both in aqueous solution (by 12% or more) and plasma (by 20% or more) whichever scan was used, and this interference was not decreased when the chloroform extract was washed with 10 mol/liter HCl (5) or phosphate buffer (0.5 mol/liter, pH 7.4) (6) before scanning by Broughton's or Jatlow's method.

Phenytoin at the grossly toxic concentration of 40 mg/liter caused a 15% underestimation of phenobarbital at a concentration in plasma of 18 mg/liter and a 5% underestimation at a phenobarbital concentration in plasma of 15 mg/liter.

Phenytoin, primidone, carbamazepine, and sodium valproate [2-propylpentanoate] had little or no effect on the estimation of barbiturate in aqueous solution by use of either scan.

When pooled patient's serum or plasma was extracted and examined by our standard GLC method (3), we observed no interference if ethosuximide, sulthiane, or sodium valproate was added to samples in vitro, and we have found no significant difference in the recoveries of phenobarbital, phenytoin, and primidone in the presence or absence of these drugs. The calculated analytical recoveries of drugs range from 98 to 99% for phenobarbital, 80 to 85% for phenytoin, and 65 to 70% for primidone under the conditions of these experiments.

The technique of GLC is now widely recommended for estimating phenobarbital in serum (7, 8) and our experience also suggests that this method should be used where possible, since there seem to be considerable disadvantages in assaying phenobarbital by spectrophotometric methods when patients are receiving multiple drug therapy.

References

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Stability of Plasma Lipoproteins for Polyacrylamide-Gel Electrophoresis

To the Editor:

With the increasing prevalence of atherosclerotic heart disease, interest in lipoprotein electrophoresis has also expanded.

The Ames Co. markets a quick and easy method for qualitative phenotyping of lipoproteins (1), which I have had the opportunity to test. The company validates storage of plasma samples at 4 °C for several weeks; this is not in accord with our experience (2), and I present results of a short stability test.

Blood was sampled from patients who had fasted for 10 h, stabilized with 8 mmol of K$_2$EDTA per liter of blood (the