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 at 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-ethylacetylurea] 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.

Sulthiame [BPC name; Sulthiame, WHO name] gave rise to the greatest interference, producing gross distortion of the ultraviolet scans (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 9% 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, sulthiame, 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 advantages 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 (7), 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
Table 1. Changes on Storage

<table>
<thead>
<tr>
<th>Storage temp, °C</th>
<th>Lipoprotein type</th>
<th>After 0 days</th>
<th>After 4 days</th>
<th>After 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 normal</td>
<td>7 normal</td>
<td>4 normal</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2 type IV</td>
<td>1 doubtful, 2 type IV</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>1 no polymerization</td>
<td>2 type IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>3 type IV</td>
<td>4 no polymerization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen</td>
<td>2 normal</td>
<td>2 normal</td>
<td>2 type IV</td>
<td></td>
</tr>
<tr>
<td>Frozen</td>
<td>4 type IV</td>
<td>4 type IV</td>
<td>4 type IV</td>
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</table>

reason for adding this volume of EDTA was that we previously had many samples to coagulate when using the amount recommended in reference 3. The samples were centrifuged within 30 min after the venipuncture, and the plasma was pipetted into polyethylene tubes, 70 mm long. To those that were to be stored at room temperature was added 1 g of NaCl per liter of plasma, as a bacteriostat, because bacterial growth can disturb the analysis (3). The tubes were corked and stored at the respective temperatures shown in Table 1. The lipoprotein analyses were performed within 30 min of removal of samples from the refrigerator or freezer (thawing at room temperature).

Besides the apparent change of type of lipoprotein pattern shown in the table, storage of the plasma samples at room temperature resulted in a number of them being unfit for analysis, because the loading-gel, after mixing with the plasma samples, would not polymerize. In the table those cases are marked as “no polymerization.”

I conclude that the information given by Ames Co. concerning the stability of plasma samples for lipoprotein electrophoresis is incorrect, because the plasma samples respond unpredictably with respect to the stability of the lipoprotein pattern, although these results are in contrast with the findings of Frajola (4). I recommend on the basis of my results a storage time of no longer than four days at 4 °C and that specimens not be stored in the freezer or at room temperature.

The method is suitable for qualitative typing of lipoproteins.

References
2. Dyerberg, J., Lipoproteinemia i plasma, thesis from the Faculty of Medicine, University of Copenhagen, September 28, 1972.

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Suggested Modification of the Definition for Definitive Method

To the Editor:
The method in reference methodology to which all others are referred is termed “definitive” and has been expected to produce results as near as possible to perfection; it has been defined in the literature on two occasions as follows:

“A technique for measuring the concentration of a given substance in samples of a particular biological material which (irrespective of the total cost involved) gives results which are accepted as the nearest attainable to the true values and the accuracy of which can be clearly proved on theoretical grounds” (1).

“A method which, after exhaustive investigation, is found to have no known source of inaccuracy or ambiguity. The result it gives is termed definitive value and is the best known approximation to the true value” (2).

Like infinity, neither definition describes an attainable entity: in the first, the term “nearest attainable” describes the quality of a result that is a function only of the effort expended, and in the second “no known source of ambiguity” is unsatisfying, since it implies absolute perfection, including absolute knowledge of the primary standards used.

Discussion at recent meetings in Europe [previously reported briefly in this journal (3)] has indicated that, if the specifications for definitive techniques are to be practical, some modification of these definitions is required. The problem discussed at the meetings involved the setting up of reference methodology for steroid measurements in blood and urine. It was clearly evident that the required definitive methods would make use of isotope dilution–mass spectrometry, and therefore, normal and isotopic steroid Standard Reference Materials (SRM’s) would be needed.

The degree of purity necessary for these SRM’s was considered, since it was realized that the highest possible degree of purity was not necessary, and in any case a “highest possible” requirement could involve unending effort and cost. This question was examined in the context of the measurement system for each steroid, that is, with view to the relationship of performance of the definitive method to the reference method and of the latter to acceptable routine methods, recognizing (a) that the SRM would provide the base for accuracy for the definitive method, (b) that usually a lower accuracy of the reference method would be observed when the two methods were interrelated, and (c) that the major purpose of establishing a measurement system for each steroid would be to have an accuracy base on which to link patients’ sample values provided by different field methods. Thus the individual SRM purity requirements should be back-calculated from an estimate of the maximum inaccuracy acceptable for a field technique to give results having clinical usefulness at medically significant concentrations. As such estimates inevitably elicit considerable discussion—since much of the criteria being considered is subjective—the values suggested at the European meetings have been published for criticism by a wider audience (cf. 3).

Consideration of the approach indicates its reasonableness for viewing the requirements for definitive measurements in clinical chemistry generally, and we wish to propose a modification of the quoted definitions accordingly, as follows:

A definitive method is one that, after exhaustive investigation, provides analytical results that are accurate, systematic errors, to the extent required for the intended end-use(s). The resulting accuracy can be specified and proved on theoretical and experimental grounds, usually from first principles. The result obtained is termed the definitive value and is the best approximation to the “true value.”

At a meeting to be held in Atlanta, Georgia in November of this year, the National Bureau of Standards will propose for discussion the following alternative and more succinct definition:

“A definitive method is an analytical