Table 1. Analysis of Variance for Incubations at Five pH Values, and at the Three Most Common pH Values

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean squares</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>28</td>
<td>6.47681</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Five pH values</td>
<td>116</td>
<td>0.00355</td>
<td>3.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH duplication</td>
<td>145</td>
<td>0.00115</td>
<td>0.95</td>
<td>ns</td>
</tr>
<tr>
<td>RIA duplication</td>
<td>290</td>
<td>0.00120</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>28</td>
<td>5.64902</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three pH values</td>
<td>58</td>
<td>0.00165</td>
<td>1.42</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>pH duplication</td>
<td>87</td>
<td>0.00130</td>
<td>1.01</td>
<td>ns</td>
</tr>
<tr>
<td>RIA duplication</td>
<td>174</td>
<td>0.00129</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

df, degrees of freedom; ns, not significant.

The pH optimum of plasma renin thus appears to have a rather broad range. The mean of the 12 pH optima reported in references 1 through 12 is 5.80 (SEM 0.07), with a 95% confidence interval of 5.66 to 5.94, which agrees with the optimum of 5.70 to 6.00 we found. The molarity of the HCl solution used to deliver the hydroxyquinoline can be adjusted such that in most instances the addition of 10 μL to 0.5 mL of plasma will result in a pH within the optimal range. The pH of samples remaining outside this range is corrected by adding the citrate buffer, which usually makes a final pH adjustment with acid or base unnecessary.

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References


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Epileptic Children Being Treated with Carbamazepine

To the Editor:

Lately, some authors have reported a possible effect of the enzyme-inducing antiepileptic drugs phenobarbital, carbamazepine, and phenytoin on α1-acid glycoprotein (AAG) concentrations in serum (1–3). The results are contradictory: some authors observed an increase of AAG concentrations in patients on mono- and polytherapy with these drugs (1, 2), others found no modification in patients on monotherapy but a decrease in patients on polytherapy (3). All reported data were obtained in adult populations, and no data, to our knowledge, are available on this effect in children.

Here we report results of a study conducted on 39 epileptic children (ages four months to 12 years, mean ± SD = 5.1 ± 3.5 years, 12 girls) treated for more than one month with a stable dose of carbamazepine, and 38 apparently healthy sex- and age-matched controls (ages two months to 12 years, mean 5.6 ± 3.7 years, 16 girls). Carbamazepine concentrations in serum, as determined by liquid chromatography (4), were in all patients between 1.3 and 11.0 mg/L (mean 5.4 ± 1.9 mg/L). Albumin concentrations were similar in both groups (36.9 ± 2.9 g/L vs 38.4 ± 3.6 g/L). AAG in serum was determined by radial immunodiffusion (Seward Laboratory, U.K.). AAG concentrations were found to be significantly higher in carbamazepine-treated patients than in controls (0.96 ± 0.35 g/L vs 0.73 ± 0.19 g/L, p <0.001, Mann–Whitney test). Moreover, seven of our 39 patients had AAG values exceeding the normal value established in our laboratory (0.47–1.19 g/L). We saw no correlation between AAG and carbamazepine concentrations.

These data agree with the increase in AAG concentrations in carbamazepine-treated epileptic adults found by Tiula and Neuvonen (2). However, in evaluating our results, it should be noted that a modification of serum AAG due to epilepsy itself cannot be excluded.

References

Improved Substrate-Labeled Fluorescent Immunoassay for Theophylline in Dried-Blood Spots on Filter Paper

To the Editor:

We previously described a substrate-labeled fluorescent immunoassay for measuring the theophylline concentration in filter paper dried-blood spots (DBS), suitable for at-home therapeutic drug monitoring, based on use of the Ames/Miles "Fluorostat" instrument, which involved manual additions of reagents (Ames TDA) and polyclonal antibodies (1). Recently, the introduction of an automated and programmable fluorometer (the Ames/Gilford "Optimate," which more than halves TDA reagent consumption) and monoclonal TDA reagents has coincided with a change in the filter paper available for the assay. We therefore studied the effect of these changes on the original procedure and report a modified method suitable for the assay on both the manual and the automated analyzers.

Capillary DBS (y) and plasma samples (x), collected from the same patient at the same time, were assayed for theophylline by using the Fluorostat analyzer, monoclonal TDA reagents, and the original protocol (1). The relationship between the two sets of results (n = 38) was highly significant (r = 0.9755, y = 0.9127x + 0.28), but the slope was 0.13 greater than that established for assays with the polyclonal antibody.

To explain this difference, we investigated the pH of the assay reaction mixtures, and found the DBS mixture to be significantly less basic (pH 7.90, SD 0.018, n = 20) than the plasma mixture (pH 8.27, SD 0.017, n = 15). To have the pH in the DBS assay approach that of the serum assay we decreased the volume of reagent used to elute the DBS disk (5'-sulfosalicylic acid, 50 g/L) to 270 µL and, after elution, combined 200 µL of the eluate with 200 µL of the manufacturer's concentrated bicine assay buffer. This modification resulted in a reaction mixture having a pH of 8.20 (SD 0.017, n = 15) and produced the 51-fold dilution of patient's sample required for the assay (the 6-mm DBS disk of PKU-31 paper, now distributed by Whatman Ltd., contains 11.0 µL of whole blood). The relationship between theophylline in DBS (y) and serum samples (x) by this modified procedure remained highly significant (n = 30, r = 0.9971, y = 0.8547, x = 0.08), and the slope was nearer to our original result. The pH difference in the reaction mixture between the serum and DBS assays had also existed in our original procedure involving the polyclonal reagents but apparently had had no effect. We suspect the monoclonal reagents, unlike the polyclonal, are pH sensitive but cannot confirm this because the polyclonal reagents are no longer available. To automate the DBS procedure for the Ames/Gilford Optimate we programmed the analyzer to "re-use the plasma dilution" (option 16 of the theophylline assay program). The diluted eluate (obtained as described above for the modified Fluorostat procedure) is placed in the dilution cup, and the assay is completed by proceeding as pre-programmed by the manufacturer for the plasma assay. The relationships obtained (2) validated the Optimate results (Table 1).

The therapeutic range for serum theophylline in asthma patients is typically 10 to 20 mg/L; the equivalent therapeutic range for capillary DBS is 8.6 to 17.1 mg/L. Other laboratories should determine their own relationship for serum/DBS values, which will depend on the characteristics of their particular punch and batch of filter paper.

DBS assay reproducibility was adequate for clinical purposes. The modified Fluorostat procedure gave within-batch CVs (n = 10) of 4.6% and 4.3% and between-batch CVs (n = 10) of 7.1% and 6.9% for DBS theophylline concentrations of 9.2 and 19.8 mg/L, respectively. The Optimate procedure gave within-batch CVs (n = 10) of 5.1%, 6.6%, and 5.2% for DBS theophylline concentrations of 6.9, 11.9, and 18.8 mg/L, respectively, and between-batch CVs (n = 12) of 6.9% and 5.8% for DBS theophylline concentrations of 11.7 and 23.4 mg/L, respectively.

The advantages to the clinician of using capillary DBS samples that can be collected and mailed from the patient's home to the laboratory for the therapeutic drug monitoring of theophylline and phenytoin in children, adults, and the elderly have been described elsewhere (1, 4–6). The automated Optimate analyzer offers the laboratory many advantages. Reagent and buffer additions as well as fluorescence measurements are automatically performed and timed, decreasing operator involvement and hence assay cost. Fluorescence is read earlier—4.5 min vs 20 min on the Fluorostat—thereby shortening assay time. Nonspecific fluorescence is also minimized. Consumption of antibody/enzyme and fluorogenic drug reagent is decreased from 50 µL to 20 µL per test. This is important when determining profiles, in which duplicate samples typically are collected when peak and trough concentrations of a circulating drug are expected, or when the patient is likely to suffer symptoms (e.g., the "early morning dipper").

Storage of standard-curve data (typically for at least three to four weeks) also decreases reagent consumption because most assay batches will then consist of quality-control and patients' samples only. The Optimate analyzer has so far performed reliably, has been well accepted by laboratory personnel, and thus seems well suited to monitor theophylline therapy with either conventional plasma samples or dried spots of capillary blood.

References
