Dibucaine Number Measured with the Ektachem System, Giampalo Cattazzo,1 Carlo Franzini,2 and Michele Rettodini31 Ospedale Filippo Del Ponte, 21100 Varese, VA, Italy; 2 Ospedale Luigi Sacco, 20157 Milan, MI, Italy (author for correspondence); 3 Ospedale USL 24, 37039 Tregnago, VR, Italy)

The catalytic activity of cholinesterase (CHE, acetylcholine acetylhydrolase; EC 3.1.1.8) in serum and its sensitivity to inhibition by dibucaine are frequently measured to detect carriers of inherited "atypical" variants of the enzyme. After anesthesia, prolonged apnea, caused by failure to hydrolyze succinylcholine, may result from the presence of such variants, which are resistant to dibucaine inhibition, whereas the normal enzyme is sensitive (1).

Recently, Eastman Kodak Co. (Rochester, NY) developed a slide for measuring serum cholinesterase in the Ektachem system, based on the method proposed by the German Society for Clinical Chemistry (2). We have developed a procedure for determination of the dibucaine number (DN) with the Ektachem system, in which we use such a slide to analyze serum to which we have added an adequate amount of dibucaine.

Sera for analysis were obtained from routine laboratory specimens. Dibucaine was from Sigma Chemical Co. (St. Louis, MO). CHE activity was measured, according to the dry-chemistry multilayer film principle, with a Kodak Ektachem 700 XRC system and Kodak Ektachem clinical chemistry slides for CHE. CHE was also measured with a conventional liquid-chemistry method, with butyrylthiocholine as the substrate and dithiobis(nitrobenzolate) as the indicator. These latter reagents were obtained as a kit from Boehringer Mannheim (Mannheim, Germany), and the method was run at 37°C with a Cobas Mira S automatic analyzer (F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Statistical analysis of the results was by conventional parametric tests; results from methods comparison were assessed by means of nonparametric linear regression (3, 4).

Cholinesterase activity was measured in 80 serum samples (activity from 1.38 to 11.76 kU/L), with the dry-chemistry method (dependent variable) and the conventional liquid-chemistry method (independent variable). Linear-regression analysis of the results gave: \( y = 0.809x + 0.01 \) kU/L (95% limits of the slope: 0.792–0.822; 95% limits of the intercept: −0.07–0.15 kU/L; standard error of estimates: 0.36 kU/L; \( r = 0.996 \)). Both methods proved linear in the interval from 1.5 to 12.5 kU/L.

Residual CHE activities, measured with the liquid-chemistry method in the presence of dibucaine, added to the same final concentrations either to the reagent or to the sera, were comparable. Sera included in this experiment were from normal subjects (12), liver patients (6) and variant carriers (4); final dibucaine concentrations varied from 0 to 250 \( \mu \)mol/L. Addition of dibucaine to serum caused considerable turbidity, because of dibucaine precipitation, but this disappeared by diluting the serum in the reagent 101-fold. Possible effects of residual turbidity were ruled out by using kinetic measurements at two wavelengths (406 and 550 nm).

Residual CHE activity was then measured with the Ektachem system, in the presence of dibucaine added to various sera in concentrations from 0 to 20 mmol/L. The results (Figure 1) were used to select the value of 12.5 mmol/L as the concentration of dibucaine in serum to be used for the routine measurement of DN with the Ektachem system. The suggested procedure is: mix 200 \( \mu \)L of serum with 10 \( \mu \)L of dibucaine aqueous solution, 262.5 mmol/L; using the Ektachem machine and slides, measure the residual CHE activity (RA) in the mixture and the total CHE activity (TA) in the undiluted serum; compute the dibucaine number (DN) as follows: DN = 100 – (RA/TA) × 105.

The DN was measured in duplicate in several sera with the suggested procedure and with the conventional method. Results were as follows:

<table>
<thead>
<tr>
<th>Samples</th>
<th>Conventional method</th>
<th>Ektachem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>n Mean ± SD</td>
<td>n Mean ± SD</td>
</tr>
<tr>
<td>Normal</td>
<td>26 81 ± 2</td>
<td>80 83 ± 2</td>
</tr>
<tr>
<td>Liver patients</td>
<td>8 78 ± 2</td>
<td>8 77 ± 2</td>
</tr>
<tr>
<td>Variants, heterozyg.</td>
<td>6 55 ± 3</td>
<td>8 60 ± 4</td>
</tr>
<tr>
<td>Variants, homozyg.</td>
<td>6 32 ± 4</td>
<td>9 41 ± 3</td>
</tr>
</tbody>
</table>

* Dibucaine in the reagent, final concentration 250 \( \mu \)mol/L.
* Dibucaine added to serum, final concentration 12.5 mmol/L.

Within-run imprecision of DN measurement (CV, from duplicate measurements) ranged from 3.8% (low-activity sera, high DN, low residual activity) to 1.5% (high-activity sera, high or low DN).

The aim of our work was to select the analytical conditions such that we could use the Ektachem system to obtain DN values comparable with those measured conventionally. This goal has been acceptably approached, although not completely achieved. Nevertheless, the tabular data above show the complete clinical usefulness of the results produced by the suggested procedure. Changes in the
protein concentration of the sample may have a variable effect on the analytical accuracy of the Ektachem systems in the assay of different components (5); to stay on the safe side, we developed the protocol to provide low preanalytical dilution of the sample (1/1.05). The turbidity appearing after the addition of the dibucaine solution to sera had no appreciable effect on the measurement.

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References