necessary to solve this problem.

Results of high-M_{r} AP measurement in the selected sera lead us to conclude that our simple gradient method is sensitive in detecting hepatic disease, and the method can even be used in laboratories that lack experience in liquid chromatography.

References

Physiological Changes in Human Erythrocyte Cholinesterase as Measured with the “pH-stat”

J. A. García-López and M. Montiel

Cholinesterase activity in human erythrocytes was determined in 1903 blood samples by the “pH-stat” method. Differences in activity were examined as a function of sex, age, and pregnancy. Reliability intervals for the population average and approval or normality intervals for individual values were established. Sex- and age-related differences were very significant.

Additional Keyphrases: sex- and age-related differences · reference interval · pregnancy · organophosphate pesticide toxicity

The main function of acetylcholinesterase (EC 3.1.1.7; AChE) is to inactivate the neurotransmitter, acetylcholine, thereby making possible the transmission of the nerve impulse. This enzyme is found in nerves, neuromuscular junctions, and erythrocytes (1), in erythrocytes as a glycoprotein with a carbohydrate/protein ratio of 0.16. Glucose, galactose, mannose, glucosamine, and sialic acid are the main constituent sugars and phosphatidylserine and cholesterol the main lipid constituents (2). Its physiological function in erythrocytes is unknown, although it has been pointed out (3) that it takes part in the ionic exchange between erythrocytes and the surrounding medium.

Acetylcholinesterase activity in erythrocytes has not been studied extensively, although it is the best indicator available to assess the degree of exposure to organophosphate pesticides (4) used in agriculture. Our aim here is to present data on normal reference intervals for AChE, and on some physiological variables affecting them.

Materials and Methods

We analyzed 1903 samples of venous blood, selected at random from collections made during a year in different laboratories in the province of Granada, Spain. Blood was collected by venipuncture, into tubes that contained EDTA as anticoagulant, and the specimens were taken to the laboratory in refrigerators at 4°C and prepared for assay within 48 h.

To determine AChE activity in the same volume of erythrocytes in all samples (from the hematocrit value as determined by the microhematocrit method), we washed a given volume of blood three times with isotonic saline and diluted the cells to 2 mL with isotonic saline to obtain a hematocrit of 45% for each sample.

To release the enzyme from the stroma of the erythrocytes, we treated samples with ultrasound (MSE 1300 sonicator, ¼-inch titanium probe) at a frequency of 45 to 60 cycles per second, applying three 30-s ultrasonic bursts at 1-min intervals. During this sonication, the vials containing the samples were kept in an ice bath. Then, 0.5-mL samples were lyophilized and stored at 4°C. At the time of analysis, each sample was rehydrated with 0.5 mL of distilled water and diluted 10-fold with 4.5 mL of isotonic saline.

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Received December 31, 1987; accepted May 27, 1988.
AChE was determined by the "pH-stat" method (5) in a FHM 62 instrument (Radiometer America, Inc., Westlake, OH 44146). This technique is based on automatic neutralization with 1 mmol/L sodium hydroxide of the acetic acid released in the enzymatic hydrolysis of the 0.11 mol/L acetylcholine chloride substrate at a constant pH of 8 and a temperature of 37 °C. AChE activity (U/L) is given in micromoles of hydrolyzed substrate per minute per liter of erythrocytes.

Student's t-test, the Bonferroni test, and analysis of variance were applied to average values for activity resulting from grouping the samples by age, sex, and pregnancy before confirming the equality of population variances by means of the test of Snedecor (6).

Results

Table 1 shows mean values for AChE activity in cells from men and women by age. For both sexes the population sample was divided into seven 10-y age groups. Subjects >60 y of age are included in the last group. Average values for AChE activity for pregnant and nonpregnant women are also indicated. In the group of nonpregnant women, only data from those older than 10 y of age were considered.

The study of sex-related differences in AChE activity (Table 2) was done separately in subjects older and younger than 10 y of age because significant differences in AChE activity were found in these two groups. Only nonpregnant women were considered in the case of women >10 y of age because higher activity values were found for pregnant women.

We calculated 95% confidence intervals for the population mean and for normality intervals (Table 3) for each of the five population groups studied.

Discussion

The availability of normal values for AChE activity is of particular interest in the prevention and diagnosis of toxicity caused by organophosphate pesticides.

Of the various methods described for determining AChE activity, the pH-stat method has proved very useful because of its speed, sensitivity, and accuracy (7). Sample preparation for analysis was carried out within 48 h of venipuncture, during which time AChE activity remained stable (8) and there were no symptoms of hemolysis (9). The sonication process per se does not bring about any significant change in AChE activity (10).

At the same time that daily measurements of the samples were taken, we also measured the AChE activity of a pool used as a quality control. This pool was prepared under conditions identical to those described for individual samples. The quality control system used was that of mean-value control charts, the error limit being the mean ±2 standard deviations. This interval was calculated after carrying out the first 30 determinations of the pool, giving 3714–3190 U/L with a mean value of 3452 U/L and a coefficient of variation of 3.79%.

Because it was impossible to determine AChE activity on the same day that the samples were prepared, a conservation method was necessary. We chose lyophilization. With this method, AChE is stable for at least 14.5 months (11). The samples we analyzed had been in lyophilized form for less than five months.

Age. The variance analysis applied to the average activity values of the seven age groups shows that there are significant age-related differences in AChE activity at a 99% confidence level in both men and women. A further comparison of mean values in pairs shows, with 95% confidence, that the differences appear in those 10 years of age in both sexes. There are no significant differences in AChE activity in subjects >10 y of age. Thus when AChE activity is measured, all subjects >10 y of age of the same sex should be considered as a homogeneous population.

Other authors (12), with a sample of 217 children, found lower values for activity in children under four months than in adults. However, they did not find activity differences between children and adults, which can be questioned because in their sample only 30 adults were included and they saw no sex-related differences.

Pregnancy: Higher AChE activity was obtained for pregnant women as compared with nonpregnant women (P <0.001). Contrary to the results obtained in our study of cholinesterase activity in erythrocytes, other authors (13) in studies of serum cholinesterase obtained lower values for activity in pregnant women, especially during the last three months of gestation. We saw no significant differences in activity with regard to month of pregnancy or subject's age, probably because the sample was not large enough—although the same conclusions were reported by other authors (14).

Sex. We found no significant sex-related differences in AChE activity for subjects <10 y of age. Nevertheless there were significant differences in activity (P <0.001) in subjects

![Table 1. Mean Values for AChE Activity (U/L) in Men and Women by Age and Pregnancy](image)

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>&lt;10</td>
<td>123</td>
<td>8996</td>
</tr>
<tr>
<td>10–19</td>
<td>136</td>
<td>9676</td>
</tr>
<tr>
<td>20–29</td>
<td>100</td>
<td>9925</td>
</tr>
<tr>
<td>30–39</td>
<td>111</td>
<td>10432</td>
</tr>
<tr>
<td>40–49</td>
<td>133</td>
<td>10058</td>
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<tr>
<td>50–59</td>
<td>156</td>
<td>10101</td>
</tr>
<tr>
<td>60+</td>
<td>158</td>
<td>9810</td>
</tr>
</tbody>
</table>

Significance

Nonpregnant 822 9514 1817
Pregnant 52 10396 1850
Significance 822 9514 1817
P <0.001

![Table 2. Mean Values for AChE Activity (U/L), by Sex](image)

<table>
<thead>
<tr>
<th>Sex</th>
<th>&lt;10 years of age</th>
<th>&gt;10 years of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Men</td>
<td>123</td>
<td>8996</td>
</tr>
<tr>
<td>Women</td>
<td>112</td>
<td>8874</td>
</tr>
</tbody>
</table>

Significance N.S. P <0.001

![Table 3. 95% Confidence Intervals for Mean Values (U/L) and Normality Intervals for Individual Values of AChE Activity](image)

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean</th>
<th>Individual values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys &lt;10 y of age</td>
<td>8776–9216</td>
<td>6268–11725</td>
</tr>
<tr>
<td>Males &gt;10 y of age</td>
<td>9887–10067</td>
<td>7010–12693</td>
</tr>
<tr>
<td>Girls &lt;10 y of age</td>
<td>8612–9136</td>
<td>5772–11974</td>
</tr>
<tr>
<td>Nonpregnant women &gt;10 y of age</td>
<td>9421–9607</td>
<td>6705–12320</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>9881–10912</td>
<td>6008–14782</td>
</tr>
</tbody>
</table>
> 10 y of age) (4495 U/L for men and 4282 U/L for women).

Sex-related differences in AChE activity are observed if all subjects >10 y of age are grouped together and also if each age group, with the exception of those 21–30 years of age, is considered individually. These sex-related differences in AChE activity were also observed by others (15) in their studies on serum cholinesterase activity.

We conclude that 95% confidence intervals for the population mean are between 28612 and 10 912 U/L and that approval or normality intervals for individual values are between 5772 and 14 784 U/L (Table 3).

References

Automated Nephelometry of Fibrinogen: Analytical Performance and Observations during Thrombolytic Therapy
J. J. M. L. Hoffmann and M. A. L. Verhappen

We evaluated the performance of an automated nephelometric determination of fibrinogen, which is an integral part of the prothrombin time assay, in a new coagulation analyzer, the ACL-810 (Instrumentation Laboratory). Results were compared with those by a total clottable protein assay and with the thrombin clotting time assay for fibrinogen. In normal and slightly abnormal plasma, the performance of the ACL method was quite satisfactory (CV 3–10%). However, in abnormal plasma (prolonged prothrombin times because of heparin or oral anticoagulants) the accuracy of the ACL method was poor. Nor could the instrument determine fibrinogen in clearly lipemic plasma. In plasma containing high concentrations of fibrinogen degradation products (FDP), collected during thrombolytic therapy with streptokinase-containing drugs, the ACL method gave spuriously high values for fibrinogen concentration. We determined that this was mainly because of interference by intermediate FDP (fragment Y). Finally, we demonstrated that early FDP (fragment X) increased the ACL results for fibrinogen to the same extent as in the total clottable protein method and that late FDP (fragments D and E) affected the thrombin clotting time method, but not the ACL fibrinogen determination.

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Received April 5, 1988; accepted June 13, 1988.

Additional Keyphrases: coagulation · variation, source of · streptokinase · fibrinogen degradation products

Because fibrinogen plays a central role in the hemostatic system, fibrinogen determinations are frequently requested in the management of ill patients. Ideally, fibrinogen assays in the routine coagulation laboratory should be sensitive, specific, and fast. The numerous published methods for determining fibrinogen in plasma are based on different principles: heat or salt precipitation, thrombin clotting time (T), total clottable protein (2, 3), turbidimetry of clotting plasma (4), and immunological techniques. In general, the more nearly accurate assays are too laborious for routine use and the simple and fast methods lack specificity or have limitations with regard to automation.

Recently, a centrifugal analyzer fully dedicated to coagulation assays was introduced: the ACL-810, which exploits a new concept for measuring fibrinogen. As an integral part of the prothrombin time (PT) determination, 1 light scattering by the clotted plasma is measured by laser nephelometry at 632 nm and the fibrinogen concentration is calculated from the net light-scattering signal. At least in normal plasma, 1 Nontandard abbreviations: PT, prothrombin time; T, total clottable protein; TCT, thrombin clotting time; FDP, fibrinogen degradation products; XDP, cross-linked fibrin degradation products; AFBAC, anisoylated plasminogen–streptokinase activator complex; SK, streptokinase; and INR, international normalized ratio.