Temporal Intrapersonal Physiological Variability of Cholinesterase Activity in Human Plasma and Erythrocytes

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Erythrocyte and plasma cholinesterase activities were measured biweekly in one group of 22 subjects for a year and daily for three weeks in another group of nine men. The average range [i.e., (range/mean) \times 100] of activity of erythrocyte cholinesterase in men during a year was 8% and during three weeks was 5%. For plasma, the corresponding values were 25% and 12%. The average ranges for erythrocyte and plasma cholinesterase activity in women during a year were 12% and 24%. Erythrocyte cholinesterase activity varies less than do hematocrit, hemoglobin, or erythrocyte count.

Several investigators have reported on the physiological variability of cholinesterase (acylcholine acylhydrolase, EC 3.1.1.8; ChE) activity in plasma. Callaway et al. (1) found that the coefficient of variation was about 7% for eight subjects from whom eight blood samples were taken during a four-week period. Wetstone and LaMatta (2) studied 82 people for various periods of as long as five years. Although they took fewer blood samples, their study extended over a longer time; the coefficient of variation they found was 8.4%. Augustinsson (3), who reported on 141 men and 60 women who were studied for several months, did not list individual variations.

Variation in the activity of the enzyme in the erythrocyte has been less well studied. Augustinsson found it to be quite small in one subject studied for two years (3).

We report here the results of three studies of variability of erythrocyte and plasma ChE activity with time.

Methods

Subjects

Twenty-two employees of this laboratory volunteered to participate in a year's study of ChE variability. Eight of them were women and 14 were men, ranging in age from 23–67 years. On alternate Monday mornings their blood was sampled for measurement of ChE activity and on each occasion they completed a questionnaire on medications, illnesses, alcohol intake, and exposure to ChE inhibitors. No subject was present at every test period. On the average, each subject was present 23 times.

Nine healthy male soldiers, age 19–24 years, had blood drawn each working day for three weeks. They completed a questionnaire similar to that described above.

A part of the annual medical examination of employees of this military installation is measurement of their plasma and erythrocyte ChE activity. From the laboratory records so accumulated, we selected data on 422 men and 28 women employees. The criterion for inclusion in this study was that the ChE activity had been measured on two annual occasions by the same person. Complete medical information on these subjects was not available.

Analytical Procedure

Cholinesterase was assayed by a continuous-flow method, with use of Technicon AutoAnalyzer modules (4). The method measures the rate at which a colored anion is formed when thiocholine, a hydrolysis product of the substrate, acetylthiocholine, reacts with a sulfhydryl-detecting reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (5).

In the procedure, plasma and erythrocyte samples are obtained from ethylenediaminetetraacetic acid-
Table 1. Variability of Cholinesterase as Compared with Some Other Hematologic Variables

<table>
<thead>
<tr>
<th></th>
<th>14 men Mean</th>
<th>Highest</th>
<th>8 women Mean</th>
<th>Highest</th>
<th>3-week study Mean</th>
<th>Highest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte cholinesterase</td>
<td>2.1%</td>
<td>3.5%</td>
<td>3.1%</td>
<td>4.1%</td>
<td>1.5%</td>
<td>1.9%</td>
</tr>
<tr>
<td>Plasma cholinesterase</td>
<td>6.4%</td>
<td>11.3%</td>
<td>6.1%</td>
<td>11.8%</td>
<td>3.9%</td>
<td>6.4%</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>3.1%</td>
<td>5.1%</td>
<td>4.7%</td>
<td>5.6%</td>
<td>2.6%</td>
<td>4.9%</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>3.3%</td>
<td>5.0%</td>
<td>5.3%</td>
<td>10.2%</td>
<td>3.2%</td>
<td>5.9%</td>
</tr>
<tr>
<td>Erythrocyte count</td>
<td>5.8%</td>
<td>7.4%</td>
<td>6.2%</td>
<td>7.8%</td>
<td>3.1%</td>
<td>4.4%</td>
</tr>
<tr>
<td>Erythrocyte cholinesterase</td>
<td>7.9%</td>
<td>11.4%</td>
<td>12.0%</td>
<td>15.9%</td>
<td>5.1%</td>
<td>7.8%</td>
</tr>
<tr>
<td>Plasma cholinesterase</td>
<td>25.7%</td>
<td>49.5%</td>
<td>24.4%</td>
<td>40.9%</td>
<td>12.8%</td>
<td>26.1%</td>
</tr>
</tbody>
</table>

(2): about 6% for both sexes. The mean ranges of values are 25.7% for men and 24.3% for women.

The activity of erythrocyte ChE was more constant in both sexes (Table 1). The highest coefficients of variation for a single subject was 4.1%; the average coefficient of variation was 2.1% for men and 3.1% for women. The highest range for a subject was 16%, the lowest 4.5%. The averages of the ranges were 7.9% for men and 12.0% for women.

For the nine subjects monitored for three weeks, the variance (coefficient of variation or range) was not as large as that found in the group studied for a year (Table 1). Most of this group of healthy young men reported that they drank heavily on occasion in the evenings and many of them engaged in strenuous physical exercise in the afternoons. No noticeable change was apparent in their ChE activities in the days following these activities. Over the three-week period the difference between the highest and lowest erythrocyte ChE activity in a single subject averaged 5.1% and the difference in plasma ChE activity averaged 12.8%.

In these small groups there was no correlation between ChE activity and age, as has been reported elsewhere (6, 7). There was a significant (P < 0.1) difference in the plasma ChE activity of men and women (4.45 vs. 3.75 μmol/ml per minute) (2, 6–8). There was no apparent season-related change in ChE activity in plasma or erythrocytes in either sex.

Although the subjects had the usual common illnesses (e.g., “colds” and influenza) during the year, neither these nor minor medications such as aspirin or antihistamines noticeably changed their ChE activities. In the course of their work, six of the subjects handled organophosphate insecticides without a noticeable decrease in their ChE activity, but after one man hung insecticide strips in his home his plasma ChE decreased by 20% during the next six weeks (these data were not included in tabulating his variation).

We also measured the packed cell volume and the erythrocyte hemoglobin content, and counted the erythrocytes on each study day in these groups fol-

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1 Glutathione. Sigma Chemical Co., St. Louis, Mo. Stated purity >98%. Repeated assays, for different lots over several years' time, have shown excellent reproducibility.
ollowed serially. The CV's of all these were greater than the CV's of the erythrocyte ChE (Table 1). From these, we calculated the erythrocyte ChE per unit of corpuscular volume and ChE per erythrocyte, hoping to find a measure relating the ChE to the cell that would have less variance than the ChE itself. However, these ratios varied more than the erythrocyte ChE.

In the population studied annually the average change of the plasma ChE was 9.3% for men and 16.5% for women. The erythrocyte ChE changes were 6.3% and 6.7% for men and women, respectively. These are smaller than for the subjects studied at more frequent intervals, but not unexpected because only two values were available.

**Discussion**

Measurement of plasma and erythrocyte ChE activities is not routinely used as a laboratory test in clinical medicine. More commonly it is used in industrial medicine as a means of monitoring unsafe procedures when the workers are around substances that might inhibit the enzyme. A decrease from "normal" might indicate that the worker has had an as-yet-asymptomatic exposure to the chemical, possibly because of working under unsafe conditions, and should be temporarily removed from the area.

In many circumstances it is difficult to judge what constitutes a significant change from "normal" or control activity. Variability in ChE activity may be the result of several factors, including true biological change and inadvertent change in laboratory technique or laboratory error. In many laboratories the procedure is not performed routinely and technicians are relatively unfamiliar with it. Even when it is commonly done, there are factors in the laboratory procedure itself that could cause misleading results if they are not monitored carefully by one who is aware of them.

The temporal variance in plasma ChE noted in these groups was slightly less than previously reported (1–3).

The erythrocyte ChE activity varied considerably less than the plasma enzyme and appears to be relatively constant.

Not surprisingly, the subjects studied for three weeks showed less variability then did those studied for a year. The day-to-day change in erythrocyte ChE activity was rather small.

The two-sample study in the larger population was undertaken to find whether this small variability is also present in a larger, rather randomly selected population. That the mean ranges were no larger suggests that the magnitude of variability found is valid.

The applicability of these findings depends on the precision of the laboratory performing the measurements. Under the circumstances described, we conclude the following:

1. The variability in erythrocyte ChE activity in normal men and women is rather small, less than that for more commonly used hematologic measures such as packed cell volume and hemoglobin content.
2. A change in erythrocyte ChE activity of greater than 8% in a man or 12% in a woman should alert one to look for a cause, either in the individual or in the environment.
3. The plasma ChE activity is much more variable and in a period of weeks may change as much as 25–50% in an apparently healthy and normal individual. The reasons for this should be investigated further.

We are grateful to the 20 people who subjected themselves to venipuncture every two weeks for a year.

**References**