Electrochemical Determination of Hemoglobin, Hematocrit, and Hemolysis

R. K. Kobos,1 S. D. Abbott,2 H. W. Levin,1 H. Kilksan,2 D. R. Peterson,2 and J. W. Dickinson1

Novel electrochemical methods have been developed for determination of total hemoglobin, hematocrit, and detection of hemolysis in whole blood. Hemoglobin is measured through its peroxidase activity, a fluoride ion-selective electrode being used to monitor the rate of fluoride ion production from the oxidation of an organofluorine compound. Results agree well with those obtained with the cyanmethemoglobin method ($r = 0.970$). Hematocrit is determined from the ratio of the sodium ion concentrations measured with an ion-selective electrode before and after lysis of the erythrocytes. Results by this and the microhematocrit method correlated well ($r = 0.987$). Hemolysis in a whole-blood sample is detected by using an oxygen electrode to measure the oxygen released when hemoglobin in plasma is oxidized.

Additional Keyphrases: ion-selective electrodes • electrolytes • intact blood as sample

In recent years there has been a growing interest in applying electrochemical sensors in clinical diagnostics. Electrochemical methods for blood gases and electrolytes are firmly established in the clinical laboratory, and sensors for glucose, urea, and for use in immunoassays have been developed (1). Although this latter group of sensors has had little impact thus far, their importance would increase substantially if they could be used in satellite laboratory and physician's office testing ("spor"). Electrochemical sensors are ideally suited for these applications. They can be made inexpensively, require simple instrumentation, and whole-blood samples can be used directly.

Here we describe the development of electrochemical methods for two important clinical laboratory measurements that have not been readily made with electrodes: hemoglobin and hematocrit. We also report preliminary results of an electrochemical method for detecting hemolysis directly in a whole-blood sample.

Quantification of total hemoglobin in whole-blood is one of the most frequently performed clinical laboratory tests. The method recommended by the International Congress of Hematology for routine measurement of hemoglobin in blood is the cyanmethemoglobin method (2), in which the cyanmethemoglobin formed by the oxidation of hemoglobin to methemoglobin and subsequent complexation with cyanide is measured spectrophotometrically at 540 nm. We found no reports in the literature of an electrochemical method for hemoglobin determination.

Hemoglobin, like other iron proteins, exhibits peroxidase activity (3), which can be used as the basis for an electrochemical assay. Siddiqi (4) developed a novel electrochemical method for determining peroxidase activity by using a fluoride ion-selective electrode to monitor the rate of fluoride ion production from the peroxidase-catalyzed oxidation of an organofluorine compound by hydrogen peroxide. The advantage of this approach is the high selectivity and sensitivity provided by the fluoride electrode. We have extended this approach to the determination of total hemoglobin by utilizing the peroxidase activity of methemoglobin to catalyze the oxidation of an organofluorine compound, e.g., fluoramidines or fluoroanilines, by cumene hydroperoxide to produce fluoride ions, as follows:

$$\text{Organofluorine compound} + \text{cumene} \xrightarrow{\text{hydroperoxide}} \text{methemoglobin} \rightarrow \text{F}^- + \text{organic products}$$ (1)

The rate at which fluoride ion is produced, as measured with the fluoride ion-selective electrode, is proportional to the hemoglobin concentration. Selectivity is provided by the use of cumene hydroperoxide, which reacts specifically with the prosthetic group of hemoglobin, and therefore does not serve as an effective substrate for catalase or other peroxidases such as horseradish peroxidase (5). We also discuss the feasibility of developing a hemoglobin sensor utilizing a...
Hematocrit, defined as the volume fraction of blood that is occupied by the erythrocytes, is most frequently determined by a centrifugation procedure, i.e., the microhematocrit method (6). Several electrochemical methods for determining the hematocrit have been reported. Kernen et al. (7) made use of the principle that the conductance of a whole-blood sample is inversely proportional to the volume occupied by the erythrocytes. However, this method is subject to errors caused by temperature fluctuations, nonlinear calibration, and abnormally high concentrations of proteins, electrolytes, and leukocytes in plasma (8, 9). Some of these problems can be eliminated by taking the ratio of the conductance of the whole blood and the blood plasma (10), but this requires a procedure to separate the blood plasma.

Another electrochemical method for determination of the hematocrit involves adding a predetermined volume of a diluent having a known concentration of a marker ion to a known volume of whole blood (11). The concentration of the marker ion, chosen such that it is excluded from the erythrocyte volume, is measured in the blood sample with an ion-selective electrode. This measured concentration can then be used to calculate the total volume of cells: erythrocytes, leukocytes, platelets, etc. Because the total volume of erythrocytes is much greater than that of the other cells, this measurement provides an approximate measure of hematocrit. The main limitation of this approach is that it requires the precise addition of diluent to an accurately known volume of blood.

This principle can be improved by lysing the erythrocytes to effect a dilution of the marker ion. If a marker ion already present in the blood is chosen—e.g., sodium ion—no addition of reagent is required, nor is it necessary to measure the blood sample volume. The method we have developed is based on measurement of the sodium ion concentration before and after lysis of the erythrocytes. Because the concentration of sodium ion in plasma (138–148 mmol/L) is much higher than that in the erythrocytes (ca. 10 mmol/L), the effect of lysing the cells is to decrease the sodium ion concentration by an amount proportional to the erythrocyte volume. The hematocrit can thus be estimated from the ratio of the two measured sodium ion concentrations; i.e.,

\[
\text{Hct} = \frac{a(1 - R)}{a + R(b - a)}
\]

where: \(a\) is the percentage of water in the plasma, typically 93% (12, 13), \(b\) is the percentage of water in the erythrocytes, typically 65% (12, 13), and \(R\) is the ratio of the sodium ion concentration in whole blood after lysis to that measured before lysis. This relationship is derived from the definition of hematocrit and the ratio of the sodium ion concentrations, as shown in the Appendix. The derivation of this equation assumes that the intracellular sodium ion concentration is negligible as compared with the concentration in plasma.

Hemolysis, the lysis of erythrocytes, may occur for several reasons during the collection of a blood sample. The net effect is to increase the concentration in plasma of those analytes that are present in high concentration in the cells—e.g., potassium—thereby producing erroneous results. In assays involving blood plasma, hemolysis can be visually detected, owing to the presence of higher concentrations of hemoglobin. A hemoglobin concentration of 200 mg/L in plasma is visible to the unaided eye (14). However, for assays with whole-blood, such as those involving electrolyte analyzers and spot testing, hemolysis will not be detected.

Hemolysis can be detected directly in a whole-blood sample by using electrochemical techniques. Changes in the hemoglobin concentration in blood plasma provide the most sensitive measure of hemolysis because the concentration inside the erythrocytes (340 g/L) so much exceeds that in the plasma (3 mg/L). The electrochemical method mentioned above for determination of total hemoglobin is not sensitive enough for the measurement of hemoglobin in plasma. However, an oxygen electrode can measure the change in the partial pressure of oxygen in whole-blood when ferricyanide is added, oxidizing hemoglobin to methemoglobin and releasing the bound oxygen. Because ferricyanide does not enter the erythrocytes and because as many as four molecules of oxygen are bound per molecule of hemoglobin, the measured change in oxygen concentration is a sensitive measure of plasma hemoglobin and therefore a sensitive means of detecting hemolysis.

Materials and Methods

Apparatus

We measured the concentration of fluoride ions with a Model 94-09 fluoride-selective electrode along with a Model 92-02 double-junction reference electrode (both from Orion Research Inc., Cambridge, MA). Potentials were measured with a Model 130 pH/mV meter (Corning Science Products, Medfield, MA), used with a Model 7132A strip-chart recorder (Hewlett-Packard, Palo Alto, CA). Reaction-rate data were collected with a Hewlett-Packard Model 85 computer (with a Keithley Model 179A multimeter interface) or a Digital Min-23 minicomputer. Photometric measurements of hemoglobin were made with a Model 320 Spectrophotometer (Perkin-Elmer Corp., Norwalk, CT).

We measured sodium ion concentrations with a Nova IV electrolyte analyzer (Nova Biomedical Corp., Waltham, MA), hematocrit with a microhematocrit centrifuge and microhematocrit reader (International Equipment Co., Needham Heights, MA), and oxygen partial pressures with an ABL 30 blood-gas analyzer (Radiometer-America Inc., Westlake, OH). We used a sonicating probe (Heat Systems-Ultrasonics Inc., Farmingdale, NY) to lyse the erythrocytes in whole-blood samples.

Reagents

Cumene hydroperoxide (95%), saponin, hemoglobin diagnostic kit no. 525A, human hemoglobin Type IV, and human methemoglobin Type II were all from Sigma Chemical Co., St. Louis, MO. p-Fluorooaniline and p-fluoroanisole were from Aldrich Chemical Co., Milwaukee, WI. The blood controls were "Hematall C" hematology controls (low, normal, and high), from Fisher Scientific Co., Pittsburgh, PA. All other chemicals were AR grade. Solutions were prepared with distilled-de-ionized water, except for the cumene hydroperoxide solutions, which were prepared in reagent-grade methanol. Fresh, heparinized blood-specimens were obtained from volunteers at the Experimental Station.

Procedures

Hemoglobin determination. In preliminary studies we used aqueous solutions of hemoglobin, prepared in 0.1 mol/L buffer, either pH 7.4 phosphate buffer or pH 5.5 acetate buffer. Stock solutions of hydrogen peroxide, 1.0 mol/L in water, and p-fluoroanisole, 1.1 mol/L in water containing 0.2 mmol sodium fluoride per liter (final pH 6.0), were optimal.
We mixed 4.4 mL of hemoglobin solution with 1 mL of \( p \)-fluoroaniline stock solution and stirred with a magnetic stirring motor until the fluoride electrode recorded a stable potential. We then added 0.2 mL of the stock hydrogen peroxide solution and recorded the potential—time response with the strip-chart recorder and the computer. All measurements were made in polystyrene microasters at room temperature \((23 \pm 1 \degree C)\). For measurements with blood samples, we substituted a 1.5 mol/L stock solution of cumene hydroperoxide in methanol for the hydrogen peroxide solution.

We diluted blood samples and blood controls fivefold with pH 7.4 phosphate buffer, which contained saponin \((3 \mathrm{~g} / \mathrm{L})\) to lyse the erythrocytes and potassium ferricyanide \((4.0 \mathrm{mmol} / \mathrm{L})\) to oxidize the hemoglobin to methemoglobin. We then followed the same procedure as described for the preliminary studies. A two-point calibration curve was prepared by assaying the low- and high-concentration blood controls at the beginning of each series of runs. Hemoglobin was measured spectrophotometrically, based on measurement of cyanmethemoglobin at 540 nm, as directed in Sigma Technical Bulletin no. 25, with the Sigma Hemoglobin Diagnostic Kit.

We also carried out preliminary studies with a hemoglobin sensor, which included an immobilized reagent membrane. The membrane was prepared by dipping a 12.5-cm diameter piece of filter paper (no. 41; Whatman, Clifton, NJ) into a solution of methanol containing 150 mmol of cumene hydroperoxide and 150 mmol of 4-fluorooanisole or 4-fluoroaniline per liter, then drying the filter paper in an oven at 110 \degree C for 10 min. The membrane was then cut into discs 13 mm in diameter, with a cork borer. To construct a solid-state fluoride electrode, we modified the Orion electrode as previously described \((15)\), replacing the liquid internal contact of the electrode with a solid-state Ag/AgF/LaF\(_3\) contact. This modification allows the electrode to be used in an inverted position, if necessary or desired. We then placed a reagent membrane on the surface of the inverted fluoride electrode, holding the membrane in place by means of a Teflon cap, which also served as the sample cell. A miniature Ag/AgCl electrode positioned above the fluoride electrode served as the reference electrode. We added to the electrode cap 0.2 mL of aqueous methemoglobin solution, prepared in pH 7.4 phosphate buffer, and determined the rate of potential change by using the computer. The potential readings were converted to fluoride ion concentrations by using a previously prepared calibration curve. The reagent membrane was replaced after each measurement.

**Hemocrit measurements.** We measured the sodium ion concentration in freshly collected whole-blood samples with the Nova electrolyte analyzer. We also determined the hematocrit of these samples by the microhematocrit method. A second portion of each blood sample was lyed, either by sonication for 10 to 15 s or by adding 10 \( \mu \mathrm{L} \) of a 25 \( \mathrm{g} / \mathrm{L} \) solution of saponin for each milliliter of blood; we then measured the sodium ion concentration of the lysed blood samples with the Nova analyzer. Using equation 2, we calculated the hematocrit of each sample from the ratio of the sodium ion concentrations. To obtain abnormally high or low hematocrit values, we concentrated some blood samples by removing some plasma, or diluted them with isotonic saline. In addition, we assayed some blood samples with abnormally low concentrations of sodium ion, prepared by diluting with an isotonic diluent that did not contain sodium ion.

**Hemolysis detection.** We first measured the oxygen partial pressure in the blood samples with the Radiometer Blood Gas analyzer. To a second portion of the blood sample (2 mL), we added 40 \( \mu \mathrm{L} \) of a 1 mol/L potassium ferricyanide solution, then measured the oxygen pressure again. We shook the blood samples to partly hemolyze them and repeated the measurement procedure. The hematocrit of the blood samples was determined by the microhematocrit method before and after shaking.

**Results and Discussion**

**Hemoglobin Determination**

In initial optimization studies we used phosphate buffer (pH 7.4, 0.1 mol/L) to dilute commercial methemoglobin preparations. This pH was chosen to minimize sample handling, even though the pH for optimum peroxidase activity of hemoglobin is 5.3 to 5.6 \((16)\). However, some measurements were made in pH 5.5 acetate buffer. The concentration of the substrates was adjusted to give pseudo-first-order kinetics with respect to the hemoglobin concentration. Sodium fluoride was added to the \( p \)-fluoroaniline solution to give a fluoride ion concentration of 36 \( \mu \mathrm{mol} / \mathrm{L} \) in the reaction cell. This concentration of fluoride provided a stable baseline potential and a rapid return to baseline after a measurement. The change in potential from the baseline value after 15 s was used as a measure of the reaction rate.

We determined the optimized response curve for hemoglobin in pH 7.4 phosphate buffer. This curve represents the average of two calibration runs. The change in potential taken after 15 s is linearly related to the logarithm of the hemoglobin concentration between 5 and 100 g/L. The slope of the linear portion of the curve is 66.8 mV per decade, the intercept \(-12.0\), and the correlation coefficient 0.9998. The standard error of estimate, and the standard deviations of the slope and intercept are 0.796, 0.795, and 1.10, respectively. The upper concentration is limited by fluoride inhibition of the reaction \((4)\) and the inability of the fluoride electrode to follow the very rapid reaction rates. Because the hemoglobin concentration range normally is between 110 and 180 g/L, the blood sample must be diluted. At pH 5.5 the reaction rates were approximately twice those observed at pH 7.4, owing to the higher peroxidase activity of hemoglobin at the lower pH.

Hemoglobin is present in several forms in blood, primarily oxyhemoglobin, deoxyhemoglobin, and methemoglobin. A study of the relative peroxidase activity of oxyhemoglobin, formed by treatment of methemoglobin with sodium hydroxysulfite, and of methemoglobin indicated that the peroxidase activity of methemoglobin was significantly higher, by about 12% under the conditions of the assay. Consequently, blood samples were treated with potassium ferricyanide to oxidize all three forms of hemoglobin—oxyhemoglobin, deoxyhemoglobin, and carboxyhemoglobin—to methemoglobin for the measurement. Note that this method is based on the assumption that all hemoglobin variants have equivalent peroxidase activity.

Cumene hydroperoxide was substituted for hydrogen peroxide in the blood assays. Hydrogen peroxide is rapidly destroyed in blood by the enzyme catalase \((EC 1.11.1.6)\). The use of cumene hydroperoxide also increases the specificity of the hemoglobin determination because it is a poor substrate for other peroxidases that may be present in blood \((5)\). The change in potential after 30 s was used as a measure of the reaction rate.
Figure 1 shows a comparison of the new electrochemical method and the cyanmethemoglobin spectrophotometric method. A least-squares analysis of the data indicates that the agreement between the methods is good. The standard error of estimate and the standard deviations of the slope and intercept were 0.584, 0.0616, and 0.861, respectively. The values for the electrochemical method represent the mean of four determinations; the spectrophotometric values are the average of three. This study was meant only to demonstrate the feasibility of measuring hemoglobin in whole-blood samples by using the electrochemical method. Further analysis of clinical samples is required for the performance of the method to be fully evaluated.

Table 1 summarizes the within-run precision of the hemoglobin determinations. In these measurements, the cumene hydroperoxide was injected manually, and the potential reading was taken at a time when the potential was rapidly changing. Consequently, small errors in the time of injection result in appreciable variations in the measured potential readings. The precision could be improved by automating the sample injection, and further diluting the sample in order to reduce the reaction rates. The reaction rates also could be decreased by substituting the less-reactive compound 4-fluoroanisole for the 4-fluoroaniline substrate. More careful control of the temperature would also be expected to improve the precision of the rate measurements.

This hemoglobin method could be readily incorporated into a flow stream. However, for seror applications a disposable hemoglobin sensor may be more desirable. Figure 2 shows preliminary results of the response of a hemoglobin sensor in which an immobilized reagent membrane is used. The sensor responds to hemoglobin in the concentration range of 10 to 100 g/L. However, the reproducibility of the measurements is poor. Further study of the preparation of reproducible reagent membranes and an inexpensive fluoride ion-selective electrode are required if a disposable hemoglobin sensor is to be developed.

Hematocrit Determination

Figure 3 compares results by the electrochemical and microhematocrit methods. A least-squares analysis of the data indicates that the agreement between the methods is excellent. The standard error of estimate, and the standard deviation of the slope and intercept are 1.41, 0.0296, and 1.23, respectively. The hematocrit values given for both methods represent the mean of three determinations. The results shown were obtained with use of saponin to lyse the erythrocytes. Comparable results were obtained when the cells were lysed by sonication. However, the use of detergent to lyse the erythrocytes may be preferred, because the leukocytes can be protected from damage by using a cross-linking agent (17). Consequently, only the erythrocyte volume would be measured.

Table 2 shows the within-run precision of the electrochemical hematocrit method. Measurements must be made with fresh blood samples, because sodium ions equilibrate across the erythrocyte membrane with time, resulting in

Table 1. Within-Run Precision for Electrochemical Hemoglobin Determinations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hb, g/L</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low control</td>
<td>56.2</td>
<td>4.1</td>
<td>7.28</td>
</tr>
<tr>
<td>Normal control</td>
<td>128</td>
<td>13.8</td>
<td>10.8</td>
</tr>
<tr>
<td>High control</td>
<td>161</td>
<td>11.4</td>
<td>7.08</td>
</tr>
</tbody>
</table>

n = 8 each.

Table 2. Within-Run Precision for Electrochemical Hematocrit Determinations

<table>
<thead>
<tr>
<th>Hematocrit, %</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>41.2</td>
<td>0.173</td>
</tr>
<tr>
<td></td>
<td>46.3</td>
<td>0.169</td>
</tr>
<tr>
<td></td>
<td>28.9</td>
<td>0.264</td>
</tr>
<tr>
<td></td>
<td>54.1</td>
<td>0.478</td>
</tr>
<tr>
<td></td>
<td>39.4</td>
<td>0.991</td>
</tr>
</tbody>
</table>

n = 9 for each of five whole-blood samples.
low hematocrits.

To test the effect of low sodium ion concentrations in plasma on the electrochemical method we measured the hematocrit of blood samples that had been diluted with an isotonic diluent that did not contain sodium ion. The results (Table 3) are similar to those obtained with normal sodium concentrations; evidently abnormally low sodium ion concentrations do not affect the electrochemical method. This study demonstrates that the selectivity of the sodium ion electrode is sufficient to prevent errors due to potassium ion interference when the sodium ion concentration is lowered and the potassium ion concentration is increased after lysis of the erythrocytes.

Unlike conductance methods, the electrochemical hematocrit method described above is not affected by abnormal electrolyte concentrations. However, abnormal protein and lipid concentrations will affect the measured hematocrit, because the aqueous volume will change. Although we made no measurements with samples containing abnormal concentrations of protein or lipids, the effect can be estimated by use of equation 2. The hematocrit would be expected to change by about 0.3 hematocrit unit for every percent change in the aqueous volume of the plasma or the erythrocytes. Therefore, for a sample with high plasma protein, i.e., 90% aqueous volume, the measured hematocrit would be low by about 2 hematocrit units. If the sample is known to have a high protein or lipid concentration, this error can be corrected by using the appropriate aqueous volume in equation 2. The Waugh equation can be used to estimate the volume of plasma water displaced by proteins and lipids (18). Further evaluation of the electrochemical method with use of abnormal samples is required.

Hemolysis Detection

The change in oxygen partial pressure when potassium ferricyanide was added to a fresh whole-blood sample with no visible hemolysis was very small, 0.48 kPa, because of the low concentration of plasma hemoglobin. This low oxygen value demonstrates that the ferricyanide does not penetrate the erythrocytes. In a blood sample that was slightly hemolyzed (hemolysis was barely visible in the plasma and there was no measurable change in hematocrit), the measured oxygen pressure changed significantly, 4.04 kPa, when potassium ferricyanide was added, demonstrating the sensitivity of the present method. A severely hemolyzed blood sample (the plasma was red and the hematocrit was decreased by 2.5 units) gave an increase in oxygen pressure of 9.25 kPa when treated with ferricyanide. Further study is required to correlate the changes in oxygen pressure with plasma hemoglobin or potassium ion concentrations. However, these preliminary results indicate that the electrochemical method may provide a simple, sensitive means of detecting hemolysis directly in whole-blood samples, without the need to isolate the blood plasma.

In conclusion: we have demonstrated the feasibility of novel electrochemical methods for determination of hemoglobin and hematocrit and for detecting hemolysis in whole-blood samples. These new methods, readily compatible with conventional electrochemical electrolyte and blood-gas analyzers, give results that agree well with those obtained by methods commonly used in the analysis of normal blood samples. Further study is required to fully evaluate the new methods in abnormal samples.

This paper was originally presented at the Spring 1986 Meeting of the Electrochemical Society, Inc., in Boston, MA.

Appendix

Derivation of equation 2.

Before lysis of the erythrocytes, the sodium ion concentration of whole-blood is given by:

\[ [\text{Na}^+]_{wb} = \text{moles Na}^+/V_p \]  

(A1)

where \( V_p \) is the plasma volume and \( a \) is the percentage of water in plasma. After lysis, the sodium ion concentration is given by:

\[ [\text{Na}^+]_b = \text{moles Na}^+/V_p + bV_e \]  

(A2)

where \( V_e \) is the volume of the erythrocytes and \( b \) is the percentage of water in the erythrocytes. Taking the ratio of equation A2 to A1 yields:

\[ [\text{Na}^+]_b/[\text{Na}^+]_{wb} = aV_p/(aV_p + bV_e) = R \]  

(A3)

and

\[ 1 - R = bV_e/(aV_p + bV_e) \]  

(A4)

Dividing equation A3 by A4 yields:

\[ R/(1 - R) = aV_p/bV_e \]  

(A5)

Hematocrit is defined as:

\[ \text{Hct} = V_e/V_{wb} = V_e/(V_p + V_e) \]  

(A6)

Equation A5 is solved for \( V_e \) and the resulting expression is substituted into equation A6 to give equation 2:

\[ \text{Hct} = [a(1 - R)]/[a + R(b - a)] \]

In this derivation it is assumed that the sodium ion concentration within the erythrocytes is negligible, and that the ionic strength remains constant upon lysis, so that concentrations can be substituted for ion activities.

Table 3. Electrochemically Determined Hematocrits of Low-Sodium Samples

<table>
<thead>
<tr>
<th>[Na⁺], mmol/L</th>
<th>Electrochem.</th>
<th>Microhemat.</th>
<th>Rel. error, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>113.2</td>
<td>38.0 (0.3)</td>
<td>41.3 (0.2)</td>
<td>-7.99</td>
</tr>
<tr>
<td>115.7</td>
<td>37.1 (0.3)</td>
<td>39.5 (0.6)</td>
<td>-6.08</td>
</tr>
<tr>
<td>111.5</td>
<td>41.4 (1.2)</td>
<td>41.4 (0.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>113.6</td>
<td>40.2 (0.2)</td>
<td>40.2 (0.2)</td>
<td>0.25</td>
</tr>
<tr>
<td>113.4</td>
<td>38.9 (0.3)</td>
<td>37.9 (0.2)</td>
<td>2.64</td>
</tr>
<tr>
<td>112.5</td>
<td>40.1 (0.3)</td>
<td>38.6 (0.1)</td>
<td>3.69</td>
</tr>
<tr>
<td>115.5</td>
<td>40.7 (0.1)</td>
<td>40.1 (0.2)</td>
<td>1.50</td>
</tr>
<tr>
<td>116.0</td>
<td>39.1 (0.2)</td>
<td>38.7 (0.4)</td>
<td>1.03</td>
</tr>
<tr>
<td>116.2</td>
<td>38.7 (0.1)</td>
<td>38.1 (0.2)</td>
<td>1.57</td>
</tr>
</tbody>
</table>

*Mean and range of three determinations.  ‡Microhematocrit method (6), for comparison.

References

We have evaluated the laboratory performance and clinical usefulness of the Roche fructosamine kit. As used with an Abbott ABA 100 bichromatic analyzer, the kit response varied linearly with fructosamine concentration to 5.0 mmol/L (deoxymorpholinofructose equivalents). Interbatch precision was 4.1% and 3.6% for respective fructosamine concentrations of 3.2 and 5.0 mmol/L; intrabatch precision was 3.2% and 3.0% (fructosamine = 3.0 and 4.0 mmol/L). In 55 nondiabetic subjects all fructosamine values were <3.0 mmol/L, 95% were <2.7 mmol/L. For both fructosamine and glycated hemoglobin (Hb A1c) the 95th percentile of the reference range corresponded to approximately the 10th percentile of values observed in 108 diabetic subjects. In the latter subjects fructosamine concentrations correlated somewhat (r = 0.6504) with the Hb A1c value and for eight diabetic subjects indicated a similar degree of diabetic control over a 10-week period. From assessments of sensitivity and specificity for predicting abnormal glucose tolerance in 145 subjects, we conclude that this assay of serum fructosamine reflects diabetic control about as well as Hb A1c estimation, but neither can replace the glucose tolerance test for the diagnosis of diabetes.

Additional Keyphrases: glycated hemoglobin and glucose tolerance test compared · diabetes · colorimetry · reference interval · cutoff values

The measurement of glycated hemoglobin (Hb A1c) and serum albumin as indices of diabetic control is well established, yet the methods of measurement are often technically difficult, time consuming, and laborious (1). Recently, a manual colorimetric assay, based on the reducing activity of glycated serum proteins (fructosamine) has been reported (2-4), and a kit version of the method has been produced. Here we describe the results of laboratory and clinical studies undertaken to assess the performance and clinical usefulness of serum fructosamine measurements made with this commercial kit.

Materials and Methods

Analytical procedures: The serum concentration of fructosamine was determined by using the Roche (F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland) kit and an Abbott ABA 100 bichromatic analyzer (Abbott Laboratories, Pasadena, CA) set as follows: reaction direction, up; mode selector, rate; kinetic mode, normal; temp., 37°C; analysis time, 5 min; no. of revolutions, 4; decimal point, 0.000; dilution plate, 1.11. The kit reagent was a carbonate buffer (0.1 mol/L, pH 10.35) containing 250 mmol of nitroblue tetrazolium chloride per liter. The fructosamine assay standard (3.55 mmol/L), used as supplied by Roche, was checked against dilutions of a 10 mmol/L laboratory standard prepared by weighing 1-deoxy-1-morpholinofructose (DMF; Sigma Chemical Co., St. Louis, MO) into a matrix of human serum albumin (40 g/L in isotonic saline) as described by Baker et al. (5).

Blood samples were drawn into plain Vacutainer Tubes (Becton Dickinson, Rutherford, NJ) at various times during the day; the serum was separated within 2 h of collection and stored at 6°C. All fructosamine concentrations were determined within one week of collection.

Hb A1c was determined by a liquid-chromatographic method previously described (6); we used chromatographic equipment from Waters Associates, Milford, MA. Glucose concentrations were determined with a BM Test-Glycemie 20-800 strips and quantified with a Refolux meter (Boehringer-Mannheim, Mannheim, F.R.G.) or by a glucose oxidase method (Glu-cinet; Sclavo Diagnostics, 20092 Cinisello Balsamo—MI, Italy).

Determination of fructosamine concentrations in diabetic