Hyperglycemic Macrocytosis in Electronically Determined Mean Corpuscular Volume

Use of Three Different Automatic Cell Counters*†

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ABSTRACT

Spurious macrocytosis in electronic cell counters has been associated with hyperglycemia. The increased mean corpuscular volume (MCV) is thought to be secondary to osmotic disequilibrium between the erythrocyte and the diluent used in the automatic cell counters. Employing three different hematology cell counters, the effects of increasing concentration of glucose (400 mg per dl to 2000 mg per dl), at different temperatures and after various incubation periods were studied. In addition to macrocytosis being temperature and glucose concentration dependent, the magnitude of cell size variation also depends on the type of instrument used.

Introduction

The observation that elevated levels of blood glucose can produce spurious macrocytosis and low mean corpuscular hemoglobin concentration (MCHC) has attracted the interest of many investigators and medical societies. These workers, after ruling out other causes of high mean corpuscular volume (MCV) in the Coulter counter system, such as cold agglutinin and high leukocyte count, found that the only factor responsible for this elevated MCV was hyperglycemia. This hyperglycemic matrix effect has been corroborated by many authors in vitro and is believed not to occur in vivo. During an investigation of this phenomenon, performed on diabetic patients with a glucose level >300 mg per dl, some corroborative in vitro experiments using three different automatic cell counters were carried out. Two of these instruments, the Coulter SSr* and the Ortho ELT-8†, employ completely different principles to measure the MCV and to count the cells; the third

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‡ Coulter Electronics, Inc., Hialeah, FL 33012.
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is a recently introduced cell counter in the American market, the Sysmex CC-800.‡ Most of the reports on this topic are based on the use of Coulter counters; one report\(^1\) briefly mentioned Ortho’s ELT-8. The present study is the first one comparing the new Sysmex CC-800, the Coulter SSr and the ELT-8 and offers several possible explanations for the variation noted in the results obtained with these instruments.

**Materials and Methods**

Blood drawn in EDTA vacutainer tubes§ from apparently healthy adults was tested. Glucose\(\) in powder form was added to the whole blood in various amounts to attain the following concentrations: 400, 800, 1200, 1600, and 2000 mg per dl. Before adding the powdered glucose, the aliquots of blood were incubated at 4°C, 25°C, and 37°C for 10 minutes. As soon as the glucose was added, the tubes were inverted 10 times. These specimens were run in the following automatic cell counters: Coulter SSr, ELT-8 and Sysmex CC-800. Spun microhematocrit (micro Hct), done by standard technique utilizing a Clay Adams microhematocrit centrifuge, was determined immediately after glucose was added and after 60 minutes incubation. On one occasion the incubation time was extended to 75 min and 120 min at 4°C. A time study with the Coulter SSr was performed at 0, 5, 10, 20, and 60 minutes after glucose was added, at a glucose level of 2000 mg per dl. Temperature studies were carried out at 4°C, 25°C, and 37°C for all glucose concentrations, after 15 minutes of incubation, using all three cell counters. One final experiment consisted of the incubation of two ml of blood (with glucose concentration of 2000 mg/dl) with 2 ml of Hematall diluent¶ for 15 minutes, and determination of the MCV using the Coulter SSr.

**Results**

Glucose added to whole blood from two apparently normal, asymptomatic, control individuals up to 2000 mg per dl (in 400 mg per dl increments) produced consistent elevation of MCV and hematocrit (Hct) at 25°C and 37°C, and concomitant depression of MCHC. The mean corpuscular hemoglobin (MCH), which is Hct-independent, remained unaltered. These abnormalities in the indices were consistently seen in all three instruments (figures 1 and 2). The ELT-8, however, showed the smallest increase (about 10 percent). Interestingly, the ELT-8 showed MCV values

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§ Vacutainer Systems, Rutherford, NJ 07070.
¶ Sigma Chemical Co., St. Louis, MO 63103.
out of the normal range (80 to 96 fl) only at relatively high glucose concentrations (1200 mg per dl and up). The Sysmex CC-800 exhibited the largest increment in MCV, from a basal 88 fl (with no sugar added) to a maximum of 125 fl (42 percent increase) with a sugar concentration of 2000 mg per dl at 37°C. The Coulter SSr showed an intermediate rise in MCV of 20 percent at 37°C (figure 1).

Time study with the Coulter SSr revealed that at 25°C the MCV reached 55 percent of the maximum raise in five minutes and 82 percent in 10 minutes. At 20 minutes, it virtually reached the maximum increase (figure 3). When the temperature was 37°C, the rate of increase was faster, rising 82 percent in five minutes and reaching the maximum in 10 minutes.

The third experiment demonstrated the influence of temperature on the variation of the parameters considered. At 4°C there was no apparent difference either in the MCV or in Hct. At 25°C there was a noticeable change in the MCV, although less pronounced than at 37°C. Again, the smallest increase was seen with the ELT-8 (4 percent at 25°C, 20 percent at 37°C), and the largest increment with the Sysmex CC-800 (19 percent at 25°C, 42 percent at 37°C) (figures 1 and 2). The Hct followed essentially the same changes described for the MCV at 25°C and 37°C (figure 4).

The results of the calculated indices by means of spun microHct are depicted in table I. A predictable observation is that when the microHct was determined...
HYPERGLYCEMIC MACROCYTOSIS IN MCV

Effects of Hyperglycemia on Erythrocyte Indices Calculated by Manual Hematocrit

<table>
<thead>
<tr>
<th>Temperature</th>
<th>4°C</th>
<th>25°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Immediate</td>
<td>60'</td>
<td>Immediate</td>
</tr>
<tr>
<td>Glucose added</td>
<td>Hct</td>
<td>MCV</td>
<td>Hct</td>
</tr>
<tr>
<td>0 added</td>
<td>46.0</td>
<td>87.3</td>
<td>46.1</td>
</tr>
<tr>
<td>400 mg/dl</td>
<td>46.0</td>
<td>87.3</td>
<td>46.2</td>
</tr>
<tr>
<td>800 mg/dl</td>
<td>45.2</td>
<td>85.9</td>
<td>46.3</td>
</tr>
<tr>
<td>1200 mg/dl</td>
<td>43.2</td>
<td>82.1</td>
<td>44.7</td>
</tr>
<tr>
<td>1600 mg/dl</td>
<td>41.4</td>
<td>78.6</td>
<td>42.0</td>
</tr>
<tr>
<td>2000 mg/dl</td>
<td>40.0</td>
<td>76.0</td>
<td>41.5</td>
</tr>
</tbody>
</table>

immediately after the glucose was added, the Hct fell from approximately 46 percent to 40 percent (13 percent change) with little variation with different temperatures. After 60 minutes incubation, however, the Hct had risen back to approximately the initial level prior to the addition of glucose, around 46 percent at 25°C and 37°C. At 4°C there was no significant change after 60 minutes incubation (on one occasion the incubation time was extended to 75 and 120 minutes, with no change observed).

When blood with glucose level of 2000 mg per dl was incubated in equal proportions with Hematall® diluent for 15 minutes, the MCV came down to 85 femtoliter (fl) from the original 101 fl. This determination was done with the Coulter SSr.

Discussion

Spuriously elevated MCV determined by electronic blood cell counters caused by hyperglycemia has focused the attention of many in the recent medical literature.2,3,4,5,7,8,9,10,11,12 This raised MCV seems to be secondary to an osmotic gradient between hypertonic erythrocytes and the relatively hypotonic, virtually aglycemic, diluent used by these automatic cell counters.1,4,5,6,9,11 Most agree that the osmotic disequilibrium occurs during the initial stages of mixing the red cells with the diluent, since preincubation with the diluent makes the spuriously elevated MCV disappear.5,6,9,11

This finding was corroborated by preincubating with diluent for 15 minutes. That this macrocytosis is an artifact owing to the diluent has been demonstrated by Holt et al5 by incubating hypertonic (hyperglycemic) erythrocytes with artificially-made hyperglycemic diluent. In this way the glucose gradient between erythrocytes and diluent is eliminated, and no spurious macrocytosis is observed. Another well documented fact that this is an artifact is the absence of raised MCV when the spun Hct is used to calculate the MCV.2,5,6,7,9

By comparing the performances of two different models of automatic cell counters, the Coulter S and the Coulter S-Plus, Holt et al5 clearly demonstrated that the degree of “macrocytosis” is greater with the Coulter S-Plus. This is due to the sample cycle characteristics of the instruments. Water crosses the red cell membrane instantaneously, temporarily causing macrocytosis, whereas the T½’s for urea and glucose efflux are 7 to 10 seconds and 24 seconds, respectively. The shorter the time allowed for efflux before sizing takes place, the greater is the degree of swelling detected. The Coulter S-Plus performs red cell sizing earlier in its cycle (12 to 21 seconds) than the Coulter S (33 to 39 seconds); therefore, there is less time for osmotic equilibration.5,10
The results of these experiments are in general in agreement with those reported in the recent literature, which shows that the rise in MCV is proportional to the concentration of glucose and is time and temperature dependent. Time study revealed that the rise begins within five minutes and virtually reaches the maximum point after 10 to 20 minutes, depending on the temperature (figure 3). Temperature significantly influenced this phenomenon, being more marked at 37°C. Almost no effect is noted at 4°C; all three instruments showed ± one fl variation in the MCV at 2000 mg per dl glucose concentration when the temperature was kept at 4°C.

When spun Hct was used for MCV calculation, no elevation of MCV was noted if the Hct was determined 60 minutes after the glucose was added to the blood. If, however, the microHct was determined immediately after the glucose was added, the Hct declined an average of 5.6 percent at all temperatures tested (table I). The most likely explanation for this is that initially there was an efflux of water from the erythrocytes owing to relative intracellular hypotonicity. After some time the influx of glucose molecules inside the cells equilibrated the osmotic gradient and the Hct returned to normal values and the MCV followed the same trend. This observation holds true at 25°C and 37°C. With low temperature (4°C) there is no appreciable change after 60 minutes, and even after 75 and 120 minutes (data not shown). This is probably because low temperature decreases all metabolic and osmotic activities, therefore not allowing osmotic equilibration.

The ELT-8 was the instrument that showed the smallest change in MCV (figure 1) and Hct. Only at relatively high glucose concentrations did the MCV rise above the normal range. The most plausible explanation of this is the technique the ELT-8 employs for measurement (laser technology), and the ELT-8's very low dilution ratio (1:600), as compared to the SSr and the Sysmex CC-800 (1:50000 and 1:25000, respectively). The ELT-8's very low dilution ratio (1:600) appears to be the most important variable in its sizing of red cells since, as compared to the SSr, its time delay before sizing is shorter (table II) and its diluent has almost the same osmolality. (The osmolality of the diluents are as follows: 340 mOsm per Kg for the ELT-8; 338 mOsm per Kg for the SSr; and 268 mOsm per Kg for the CC-800.) On the other end of the spectrum, the Sysmex CC-800 showed the largest variation in MCV and Hct (figure 1), which is in all probability secondary to the same factors mentioned previously. In the SSr and the CC-500, the time delay before counting (table II) appears to play the leading role in equilibration, since both show a high dilution ratio. Hence, the changes observed in the MCV seem to represent the result of a complex interplay of these factors, in addition to the previously mentioned factors (time, temperature, glucose concentration).

Clinically, spurious macrocytosis may be determined by noting that the MCHC is low and that the MCV and MCHC normalize as the serum glucose level decreases. Realizing this should keep the clinician from pursuing an unnecessary and costly workup for macrocytosis.

In summary, this study shows that "macrocytosis" owing to hyperglycemia in electronically determined MCV is not only proportional to the concentration of glucose (up to 2000 mg per dl in our study), is temperature and time dependent, and is essentially an in vitro and
transient phenomenon, but the osmolality of the diluents, the ratio of dilution of the samples, and the time delay before counting also appear to play an important role. The ELT-8 showed the smallest increase in MCV, whereas the Sysmex CC-800 showed the largest. It is concluded, therefore, that the magnitude of the variation of the MCV is also instrument-dependent.

Addendum: After submission of this manuscript, TOA Medical Electronics (USA), Inc., informed the authors that they improved their diluent, and discontinued diluents Cellent and Cellent A, for use with their models CC-700, CC-720 and CC-800 hematology analyzer.

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References


