CONCISE REPORT

Inaccuracies Associated With the Automated Measurement of Mean Cell Hemoglobin Concentration in Dehydrated Cells

By Narla Mohandas, Margaret R. Clark, Susan Kissinger, Carol Bayer, and Stephen B. Shohet

Because of discrepancies between electronically and manually measured values of mean cell hemoglobin concentration (MCHC) encountered in studies of pathologic red cells, we studied the effect of cell water content on MCHC measurements by both methods. A series of red cell samples with varying water contents (54%-164% normal) were prepared from normal cells using the antibiotic nystatin. MCHC was then measured, using the microhematocrit centrifuge and three different electronic cell counters in common laboratory use. For MCHC values above 36 g/dl as measured by the spun hematocrit method, all three electronic counters underestimated the MCHC, with increasing error as the true MCHC increased. For MCHC values below 30 g/dl, the values from two conductivity based instruments agreed with those from the spun hematocrit method, whereas one instrument based on light scattering overestimated the MCHC. These results indicate that inaccuracies in the measured mean cell volume (MCV) of dehydrated or otherwise undeformable cells may lead to spurious values for MCHC when electronic cell counters are used.

In the initial evaluation of anemia, the mean cell volume (MCV) provides an important clue toward understanding the underlying cause of the anemia. Furthermore, because the electronic cell counters used in most clinical laboratories use the measured value of the MCV to derive the hematocrit and mean cell hemoglobin concentration (MCHC), the accuracy of these indices also depends on an accurate determination of the MCV. During the course of some experiments involving cells with very high MCHC, we became aware that MCHC values obtained from the electronic cell counter were significantly lower than those obtained from direct measurements of hemoglobin and spun hematocrit. Because the hemoglobin values were constant by the two methods, it was apparent that the discrepancy was in the values determined for the hematocrit.

To investigate this problem further, we prepared a series of cell samples in which we varied over a wide range the water content of otherwise normal cells. We determined the MCHC of the samples using the spun hematocrit, as well as three popular electronic cell counters. We have found that for cells with very high MCHC, all three electronic counters substantially overestimated the MCV, thus giving a low value for MCHC. In addition, one electronic instrument inaccurately measured the volume of swollen cells containing excess water. These results suggest that in the determination of red cell indices when appreciable deviation from normal MCV is suspected, alternative methods should be used to confirm values obtained from the electronic instruments.

MATERIALS AND METHODS

The water content of normal red cells was varied by diluting or enriching the extracellular concentration of monovalent cations (Na or K) in the presence of the permeabilizing antibiotic nystatin. Incubation with nystatin (30 μg/ml) at 0°C permitted equilibration of Na and K across the membrane at the prevailing osmotic concentration. Subsequent removal of nystatin by washing at room temperature with media having the same ion concentrations restored the normal permeability barrier. Then, when the cells were resuspended in isotonic (290 mosmole) phosphate-buffered saline (PBS, 10mM sodium phosphate, pH 7.4), they underwent shrinkage or swelling, depending on whether the osmolality during nystatin treatment was hypotonic or hypertonic, respectively. The extent of water loss or gain was consistent with the deviation from normal osmolality during ion equilibration.

After washing and resuspension of the cells, their hemoglobin content was measured using cyanomethemoglobin reagent containing 0.1% Triton X-100 to ensure complete lysis. The hematocrit was measured using 10 μl microcapillary tubes as described by Strumia et al. Increase or decrease of cell water content was further confirmed by measurement of osmotic fragility and Na and K concentrations. A further evaluation both of cell water content and population homogeneity was provided by centrifugation of the cells on discontinuous stractan density gradients over the appropriate density ranges.

Finally, samples were taken to the clinical laboratories for measurement of red cell indices on the Coulter S, Coulter S-plus (Coulter Electronics, Hialeah, Fla.), and the Ortho ELT-8 (Ortho Instrument, Westwood, Mass.) electronic cell counters. Coulter measurements were made both on 40% cell suspensions and samples prediluted into Isoton II Unopettes (Becton-Dickinson, Rutherford, N.J.).
### Table 1. Values of Mean Cell Hemoglobin Concentration and Intracellular Cation Concentrations for Normal, Hydrated, and Dehydrated Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Mean Cell Hemoglobin Concentration</th>
<th>Cell Cation Concentrations Na+ K+ meq/liter</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Spun Hematocrit</td>
<td>Coulter-S</td>
</tr>
<tr>
<td>Control untreated cells</td>
<td>34.6</td>
<td>35.2</td>
</tr>
<tr>
<td>Hydrated cells</td>
<td>31.2</td>
<td>31.1</td>
</tr>
<tr>
<td>Dehydrated cells</td>
<td>39.7</td>
<td>36.3</td>
</tr>
<tr>
<td></td>
<td>42.8</td>
<td>37.9</td>
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<tr>
<td></td>
<td>45.3</td>
<td>37.0</td>
</tr>
<tr>
<td></td>
<td>47.8</td>
<td>38.4</td>
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<tr>
<td></td>
<td>51.4</td>
<td>40.1</td>
</tr>
</tbody>
</table>

*ND, not determined.

**RESULTS**

The cells studied had water contents ranging from 54% to 164% of normal, with a corresponding MCHC range of 24–52 g/dl. While the values for MCHC obtained from the three instruments agreed with those derived from the spun hematocrit method in the normal range (33–35 g/dl), marked deviations were observed outside this range (Table 1), especially for dehydrated cells. The Ortho instrument gave somewhat higher MCHC values than the two Coulter instruments, but all three instruments underestimated the MCHC of dehydrated cells. While the Ortho instrument gave a maximum MCHC of 44 g/dl, the Coulter S gave a value of 40 g/dl compared to a manually derived value of 51.5 g/dl. Surprisingly, the Coulter S-plus did not record values above 34 g/dl, except for one sample that gave a value of 35 g/dl. Thus, all the dehydrated cells appeared virtually normal as measured by this instrument. For hydrated cells, both Coulter counters gave values that were in substantial agreement with those derived from the spun hematocrit method. However, the Ortho instrument markedly overestimated the MCHC for these low MCHC cells (Table 1). The decreases and increases in monovalent cation concentrations, as summarized in Table 1, confirmed the relative extent of water loss or gain in the treated cells.

Further independent confirmation of the abnormal water content of the nystatin-treated cells was obtained from measurement of osmotic fragility. Figure 1 shows the marked and progressive shift in osmotic fragility to lower and higher osmotic strengths, respectively, for the dehydrated and hydrated cells.

Finally, the centrifugation on stractan density gradients showed that the nystatin-treated cells were homogeneous in density. The photographs in Fig. 2 show that the entire cell population was moved to progressively higher or lower densities that were consistent with the increase or decrease in MCHC. Morphological examination of the dehydrated cells showed normal shape except for a slight flattening of the disc at very high MCHC. The hydrated cells were also smooth, progressing from thicker discs to slightly cupped cells (early stomatocytes) at the lowest

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**Fig. 1.** Osmotic fragility curves confirming the altered water content of nystatin-treated cells. Normal cells are represented by the shaded area. Progressively hydrated cells (A–D) show increased fragility with progressive displacements of the curve to higher sodium chloride concentrations. Conversely, dehydrated cells (G–K) show comparable osmotic resistance. Corresponding MCHC (spun hematocrit method) ranged from 26.9 to 50.8 g/dl.
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Fig. 2. Distribution of hydrated and dehydrated cells on discontinuous stractan gradients. Gradients consisted of 12 layers ranging from 1.070 to 1.124 g/ml density in 0.045 g/ml increments. The corresponding MCHC measured by the spun hematocrit method were 26.6, 30.1, 36.1, 39.4, 42.0, 44.3, and 49.2 going from left to right in the photograph.

MCHC. The mean cell hemoglobin content (MCH) for all the treated samples was the same as for the corresponding untreated control, indicating that no hemoglobin had been lost during cell preparation.

The MCHC measurements for 40 different cell samples are compared in Fig. 3, which shows electronically derived MCHC values versus the manually measured values for the same samples. Note the consistent deviation from the line of identity at MCHC values greater than 38 g/dl for all instruments, and the deviation below 28 g/dl for the Ortho instrument.

DISCUSSION

The availability of a systematic method for altering the isotonic water content of red cells in a controllable way has provided an opportunity to evaluate the performance of different electronic cell sizing instruments over a very broad range of cell size and hemoglobin concentrations. Because the numerical values obtained for red cell volume from these instruments are highly reproducible, electronic counters have been regarded as accurate tools for the measurement of MCV. The studies reported here show that, although indeed precise, these instruments are accurate only over a comparatively narrow range of mean cell hemoglobin concentration. Previous studies have clearly shown that reduced cell deformability can strongly influence the MCV measurement by the Coulter type instrument,\textsuperscript{5} in which the actual shape of the cells as they flow through the instrument aperture determines the measured volume.\textsuperscript{6} Thus, it is not surprising that dehydrated cells, in which high internal viscosity causes a profound reduction in deformability,\textsuperscript{7} were not measured accurately. On the other hand, even substantially swollen red cells or cells with a marked reduction in surface area to volume ratio will deform in isotonic medium.\textsuperscript{8} Thus, the fact that the Coulter counter was able to accurately measure the volume of these cells supports the idea that reduced cell deformability was the major factor responsible for the anomalies in measurements of dehydrated cells in this instrument. The Ortho instrument, which measures cell volume by analysis of light scattering, is apparently less sensitive to abnormalities in cell deformability. It is possible that its ability to provide an accurate measure of the volume of swollen cells could be compromised by the reduced refractive index of these low MCHC cells.

One might argue that variations in trapped extracellular medium might render spun hematocrits inaccurate over this wide range of cellular hydration. However, any increase in trapped medium for poorly deforming dehydrated cells would cause an overestimation of hematocrit and consequent underestimation of MCHC. Thus, the manual hematocrit measurement provides a lower limit for the true MCHC, and
the actual error for the instrumental measurements may be even greater than our results indicate. The corroborative data from measurements of cell density, osmotic fragility, and the cation concentrations further indicate that cells with high MCHC are not accurately measured by the electronic instruments.

These studies indicate that electronic sizing instruments have a limited range for accurate MCV and MCHC determination. For the vast majority of clinical samples, this range is not exceeded. However, in some infrequent hemolytic disorders for which accurate MCV data could be particularly useful, reductions in cell deformability may produce misleading and inaccurate data. For those cells with high MCHC, such as hereditary spherocytes, irreversibly sickled cells, and desiccated cells, one must be especially cautious in accepting red cell indices obtained by electronic instruments. Moreover, the value of indices obtained by such instruments in screening populations for these disorders is markedly limited.

A recent controversy has arisen concerning the clinical usefulness of the MCHC index since it shows so little variation in a large patient population. The constancy of electronically measured MCHC values among patient populations may in some cases be partially explained by the problems described above.

These studies show that electronic cell counters have a limited range of accuracy for the measurement of MCHC. This limitation usually does not interfere with the evaluation of clinical blood samples. However, in research studies involving high MCHC cells, spun hematocrits should be used to validate electronically measured MCHC values.

REFERENCES

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