Inaccuracy in Automated Measurement of Hematocrit and Corpuscular Indices in the Presence of Severe Hyperglycemia

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Because we recently observed two patients with severe diabetic hyperglycemia and spuriously elevated electronically determined hematocrit and mean corpuscular volume (MCV), we investigated the effect of hyperglycemia on two popular automated hematologic systems, the Coulter S and Ortho ELT-8. Marked hyperglycemia (blood glucose 800–2000 mg/dl) caused consistent overestimation of the electronically determined MCV compared to that derived from a simultaneous spun microhematocrit. The resultant overestimation and underestimation, respectively, of the derived values for hematocrit and mean corpuscular hemoglobin concentration may be clinically misleading. The mechanism of MCV elevation in hyperglycemia appears to be swelling of hyperosmolar glucose “loaded” erythrocytes when diluted to “isotonic” counting medium. This effect is readily circumvented by determination of a spun microhematocrit.

In most clinical laboratories, routine hematologic determinations are performed using electronic cell counting instruments such as the Coulter S and Ortho ELT-8, which are subject to a variety of inaccuracies. These instruments utilize an electronically or optically measured mean corpuscular volume (MCV) from which the hematocrit and mean corpuscular hemoglobin concentration (MCHC) are derived. The accuracy of the hematocrit and MCHC determinations are therefore dependent on the single measured value for MCV. We recently encountered two diabetic patients with marked hyperglycemia and spuriously elevated MCV and hematocrit determinations on the Coulter S instrument. These changes rapidly reverted upon in vivo correction of the hyperglycemia. To investigate this phenomena further, we studied the effect of in vitro manipulation of blood glucose on electronic determination of MCV by two popular automated cell counters.

Case Reports

Case 1

A 52-yr-old male with insulin-requiring diabetes mellitus and renal insufficiency was admitted with a clinical diagnosis of hyperosmolar nonketotic coma. Admitting laboratory values were glucose 1696 mg/dl, blood urea nitrogen (BUN) 67 mg/dl, sodium 104 meq/liter, potassium 8.8 meq/liter, chloride 70 meq/liter, and bicarbonate 15 meq/liter. Admitting hematologic values (Coulter S in duplicate) were hemoglobin 13.0 g/dl, hematocrit 47.0%, RBC 4.34 × 10^12/µl, MCV 106 µm, MCHC 27.7%. Red cells, in peripheral blood smear, were normocytic and normochromic. The patient was treated vigorously with intravenous fluids and insulin. Twenty-four hours after admission glucose was 389 mg/dl, BUN 32 mg/dl, sodium 131 meq/liter, potassium 4.2 meq/liter. Repeat Coulter S values were hemoglobin 12.3 g/dl, hematocrit 35.6%, RBC 4.23 × 10^12/µl, MCV 84 µm, and MCHC 34.7%.

Materials and Methods

Blood samples were obtained from healthy, fasting donors, using standard EDTA anticoagulant (Vacutainer). Plasma glucose was determined by autoanalyzer (Technicon). Blood glucose was adjusted in vitro by the addition of dextrose 40%. Glucose concentration was adjusted to values based on whole blood ranging from baseline (approximately 90 mg/dl) to 2000 mg/dl. Incubations were carried out at 25°C except as noted.

Spun microhematocrit was determined by standard technique utilizing a Clay Adams microhematocrit centrifuge at 10,000 rpm for 5 min. Automated electronic counting was performed using two separate Coulter S instruments (Coulter Electronics, Hialeah, Fla.) and an Ortho ELT-8 instrument (Ortho Instrument, Westwood, Mass.). Instruments were calibrated in standard manner and counts performed in duplicate on undiluted samples except as noted. Serial determinations were performed at 15, 30, and 60 min following the addition of glucose.

Results

Addition of glucose to normal blood at concentrations of 1000 and 2000 mg/dl resulted in consistent elevation of the MCV and hematocrit and depression of the MCHC as compared to control values and

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values determined from a simultaneous spun microhematocrit (Table 1). The effect was temperature dependent, appeared within 15 min of the addition of glucose and was relatively constant over 60 min (Tables 1 and 2). The addition of glucose affected only those parameters derived from the electronically determined MCV (MCV, hematocrit, MCHC). Hemoglobin, mean corpuscular hemoglobin, and red blood cell count were not affected. Predilution of hyperglycemic samples in isotonic medium resulted in rapid reversal of the effect (Table 2). Quantitative studies of the effect of glucose over a range of 90–2000 mg/dl (Fig. 1) demonstrated progressive overestimation of the MCV by both the Coulter S and Ortho ELT-8 instruments as compared with a value determined from a simultaneous spun microhematocrit. Examination of smears and wet mounts of experimental samples demonstrated no changes in erythrocyte morphology. True MCHC (MCHC determined on the basis of a spun microhematocrit) was not changed.

**DISCUSSION**

The mechanism of MCV overestimation associated with hyperglycemia is uncertain. The temperature dependence of the effect suggests it is related to glucose uptake by the red cells, a temperature-dependent phenomenon. The most likely explanation is that of osmotic disequilibrium between the glucose "loaded" erythrocytes and the relatively hypotonic counting medium. When the hyperglycemic erythrocytes are introduced into the isotonic (and therefore relatively hypoosmolar) diluent of the counting system, they presumably take up water and transiently swell. This mechanism would be consistent with the observed rapid reversal of the effect when the hyperglycemic erythrocytes are held briefly in isotonic diluent prior to counting (Table 2), permitting osmotic reequilibration. Cellular dehydration does not appear to play any role, since there is no measurable change in true MCHC even at extreme levels of hyperglycemia (Table 1).

Hyperglycemia of the degree necessary to produce this phenomenon occurs infrequently in clinical medicine but may be an occasional cause of misleading automated hematology measurements. The difficulty, if suspected, is readily circumvented by determination of a spun microhematocrit.
REFERENCES


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