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Marked interference of hyperglycemia in measurements of mean (red) cell volume by Technicon H analyzers

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Severe hyperglycemia can result in falsely high results for mean cell (erythrocyte) volume (MCV), which will also cause false results for erythrocyte indices calculated on the basis of MCV. Falsely high MCV results were obtained with the Technicon H1 and H2 analyzers and (to a lesser extent) with the Coulter T660. The H analyzers were more susceptible to this interference than was the Coulter T660. This difference in sensitivity of MCV to hyperglycemia can be explained by the use of sodium dodecyl sulfate in the Technicon erythrocyte diluent and by differences in incubation times. In severe hyperglycemia, results for MCV, mean cell hemoglobin concentration, and hematocrit obtained from electronic cell counters, especially Technicon H systems, are unreliable.

INDEXING TERMS: intermethod comparison • erythrocytes • analytical error

Hyperglycemia may falsely increase mean (red) cell volume (MCV) as measured on automated analyzers.[1] Three recent cases are illustrative, in which MCV results for patient S. and patient B. were determined with a Technicon H2 analyzer, and those for patient H. with a Technicon H1. None of these patients received a blood transfusion during the periods of observation.

Patient S. was admitted to the intensive care unit with hyperglycemia (97 mmol/L) and ketosis (pH 7.15, HCO_3^- 7.1 mmol/L). After 4 weeks the patient was discharged from the unit. At admission the MCV was 111 fL, which fell overnight to 90 fL in parallel with the decrease in blood glucose after treatment with insulin and saline (Fig. 1).

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[1] Nonstandard abbreviations: MCV, mean cell volume; MCHC, mean cell hemoglobin concentration (CHCM, measured MCHC); RBC, red blood cells (erythrocytes); MCH, mean cell hemoglobin mass; PBS, phosphate-buffered saline; and SDS, sodium dodecyl sulfate.

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Patient H. was sent to the hospital by her general practitioner because of a blood glucose of 40.2 mmol/L. At admission her glucose and MCV were respectively 64 mmol/L and 91 fL. During treatment both fell to 11.5 mmol/L and 85.5 fL, respectively.

Patient B. was also admitted to the intensive care unit with hyperglycemia (38.2 mmol/L) and ketosis (pH 6.95, HCO_3^- 1.8 mmol/L). The initial MCV (98 fL) fell to 86 fL after the blood glucose was lowered to 9.9 mmol/L.

Additional laboratory results for all three cases are shown in Table 1. Further studies were carried out in search of the underlying mechanism of this interference.

Materials and Methods

ANALYZERS

Technicon H1/H2 hematology analyzers (Bayer/Technicon, Mijdrecht, The Netherlands) determine both the size and hemoglobin content of red blood cells (RBCs) *[1, 2]*. For determination of RBC indices, whole blood is diluted 625-fold with RBC diluent (Bayer/Technicon), which contains, among other things, the detergent sodium dodecyl sulfate (SDS) and the fixative glutaraldehyde. RBCs are made spherical isovolumetrically, fixed, and measured individually by laser light scatter. Mean cell hemoglobin concentration (MCHC) and MCV are calculated from individually measured RBCs. MCHC is also calculated by the analyzer from the total hemoglobin, the RBC count, and the MCV, as are the mean cell hemoglobin mass (MCH) and hematocrit. (Measured MCHC is referred to by the manufacturer as CHCM.) Whole blood is incubated with RBC diluent for 20 s in the H1 analyzer and only 16 s in the H2 analyzer. Both analyzers measure RBC indices for 10 s.

The Coulter T660 hematology analyzer (Coulter Electronics Nederland, Mijdrecht, The Netherlands) measures blood cells by the aperture impedance method, through a decrease in conductivity when a cell passes between two electrodes, replacing a conductive diluent (Isoton III; Coulter Electronics Nederland) *[3]*. Whole blood is diluted 6250-fold, incubated for 10.5 s, and measured in three successive 4-s intervals with a 0.5-s pause between the intervals. The MCV is calculated from the three separate MCV values determined in these intervals. The

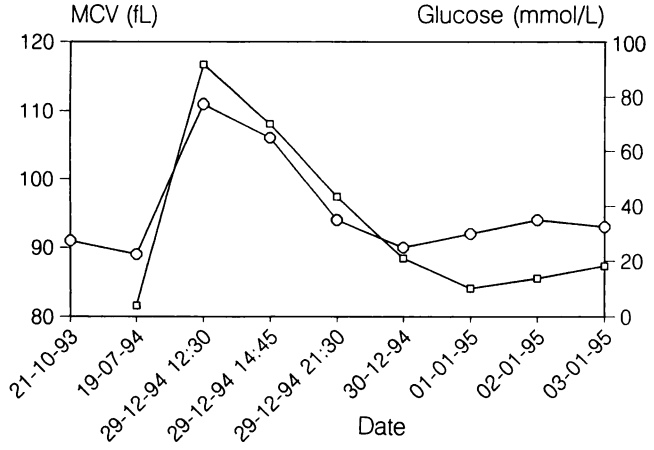

Fig. 1. Changes of glucose concentration (□) and MCV results (○) over time for patient S.

MCHC of RBCs is not measured but is calculated from total hemoglobin, RBC count, and MCV. MCH and hematocrit are also calculated from these data.

Osmolality of plasma was measured with a Wescor 5500 vaporization osmometer (Stam Instrumenten, Eerbeek, The Netherlands). Glucose in whole blood was measured with an ESAT glucose analyzer (glucose oxidase method; Depex, De Bilt, The Netherlands).

EFFECT OF HYPERGLYCEMIA ON MCV MEASUREMENTS

EDTA blood, drawn from three healthy volunteers, was divided into 1-mL portions, to which was subsequently added 100 μL of saline containing glucose (BDH, Brunschwig, Amsterdam, The Netherlands) at various concentrations. The samples were incubated at room temperature. RBC indices were determined with the Technicon H2 at 0.5, 2.5, and 8 h after addition of glucose and with the Coulter T660 at 2.5 h after the addition. Glucose was measured shortly after glucose addition and after 8 h. No sodium fluoride was added to the samples because of its effects on RBC morphology.

This procedure was repeated with blood from three volunteers at three glucose concentrations to compare Technicon H1 and Technicon H2. These samples were analyzed 2.5 h after the addition of glucose.

Table 1. Relevant laboratory results for the reported cases.

Hospitalized day	Glucose, mmol/L	MCV, fL	mmol/L Sodium	mmol/L Urea	Osmol., mosmol/kg[a]
Patient S.					
1	92	111	130	23	375
4	10.2	92	136	9.9	292
Patient H.					
1	64	91	115	24	318
2	11.5	85	130	15	287
Patient B.					
1	38	98	121	9.3	289
2	9.9	86	136	6.0	288

[a] Calculated osmolality: 2 × [sodium] + [urea] + [glucose].

INFLUENCE OF INCUBATION TIME, FIXATIVE, AND DETERGENT

To evaluate the influence of duration of incubation, cell fixation, and detergent on the effect of hyperglycemia on MCV measurements, we performed the following experiments:

Blood samples with added 56, 84, and 124 mmol/L glucose were analyzed with the Coulter T660. Mean MCV and MCV from the three separate intervals were recorded individually.

A blood sample containing 5 mmol/L glucose and with 53, 79, and 119 mmol/L glucose added, and additionally five randomly selected normoglycemic patients' samples were analyzed in RBC diluent (with glutaraldehyde) or in phosphate-buffered saline (PBS) of equal osmolality (290 mosmol/kg) containing 35 μmol/L SDS (Sigma-Aldrich N.V., Bornem, Belgium) but no glutaraldehyde. This SDS concentration is identical to that used in RBC diluent; moreover, addition of SDS did not change the osmolality. The system was operated as in routine use, according to the manufacturer's instructions, so PBS-SDS was presented to the system at the position of the RBC diluent container.

Blood samples with 5.9 mmol/L glucose and with added glucose of 56, 84, and 124 mmol/L were analyzed by the Coulter T660, with use of Isoton III diluent (which does not contain SDS) and with Isoton III plus 35 μmol/L SDS (Isoton III-SDS). Moreover, four randomly selected patients' samples and quality-control samples (Testpoint Hematology Control; Miles/Bayer) were analyzed with both diluents. The Coulter T660 was not calibrated with Isoton III-SDS.

The use of human subjects was in accordance with the Helsinki Declaration of 1975, as revised in 1983.

For statistical data analysis Student's paired *t*-test was used.

Results

Figure 2A shows the Technicon H2 results for MCV in a sample from a healthy volunteer to which various amounts of glucose were added. Just 30 min after addition of glucose a concentration-dependent increase of MCV is apparent. The decrease of MCV at lower glucose concentrations measured after long incubation times is the result of consumption of glucose by the cells (~7 mmol/L after 8 h). Fig. 2B shows the MCV and MCHC pattern (after 2.5 h incubation). The Technicon H2 system measures volume and hemoglobin concentration for every erythrocyte individually. As Fig. 2 shows, when the MCV increases, the corresponding MCHC value declines. This clearly suggests that the volume of the erythrocytes was increased, which resulted in a decrease of cellular hemoglobin concentration. The amount of hemoglobin per RBC did not change: 1848 amol ± 0.8% (data not shown).

The MCV reported by the Coulter T660 started to increase at higher glucose concentrations than those reported by Technicon H2. Fig. 3A shows the percentage increase of MCV for samples from a representative donor at various glucose concentrations. The small variations in MCV reported by the Coulter T660 at the lower glucose concentrations results from the fact that in this experiment Coulter T660 reported MCV results in integers, whereas the Technicon H2 system reported to one decimal place. The increase of MCV shown by the Technicon H1 was considerably higher for all three donors [$P < 0.01$,

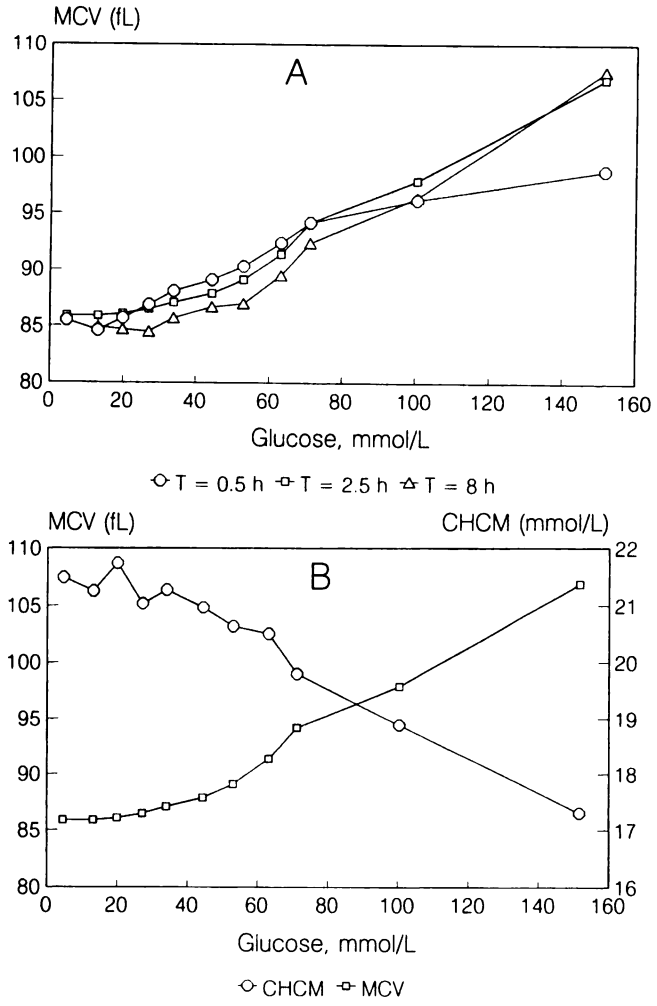

Fig. 2. (*A*) Time-dependent increase of MCV with increase of glucose concentration after various incubation periods; (*B*) measured MCV and MCHC (CHCM) 2.5 h after addition of glucose.

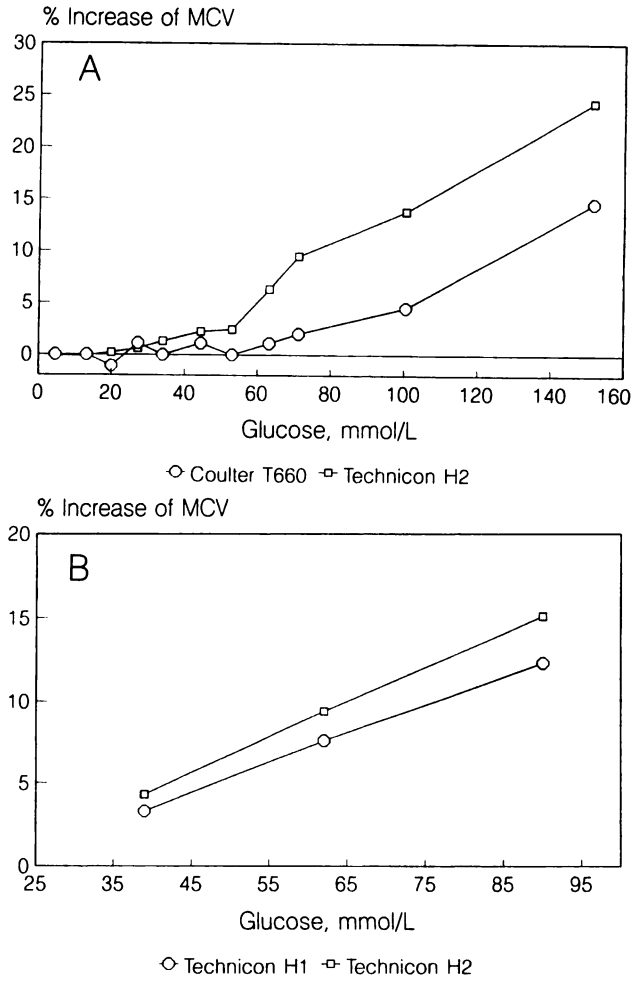

Fig. 3. Percent increase in MCV by Coulter T660 and Technicon H2 (*A*) and by Technicon H1 and H2 (*B*) 2.5 h after addition of glucose.

<0.01, and <0.05) than was observed with the Coulter T660, but was less than by the Technicon H2 (Fig. 3B). At the highest glucose concentration, the mean − 2SD of Technicon H2 MCV results was higher than the mean + 2SD MCV measured by Technicon H1 for all three donors. Student's paired *t*-test showed that this difference was statistically significant for one donor (Fig. 3B; $P < 0.05$).

The results at the three measuring time intervals of the Coulter T660 show that during the incubation time the MCV decreases ($P < 0.05$) (Table 2). This decrease of MCV was not observed when no glucose was added to the samples (n = 4) or in four randomly picked patients' samples (not shown).

We next studied the effect of the fixative agent glutaraldehyde on increases of MCV. When we used PBS-SDS instead of RBC diluent, otherwise operating the system as in routine use, results for MCV and other red cell indices were comparable with those for routine determinations made with RBC diluent (not shown). The red cell count was nearly identical for the two diluents. The system was not calibrated with PBS-SDS, which might explain a minor increase of MCV (~1.5 fL).

Figure 4 shows that when SDS was added to Isoton III to match the final concentration used in RBC diluent, the percentage increase of MCV reported by Coulter T660 for increasing glucose concentrations was considerably higher ($P < 0.05$) than the increase noted without this addition. This was observed for all three measuring intervals. The results reported by the system (i.e., the mean of the three individual results) were nearly identical to the results of the second measuring interval. In this experiment, the increase of MCV for all three measuring intervals was even higher than the results obtained by analyzing the samples with the Technicon H1. MCV results for patients'

Table 2. Results of the measuring intervals of Coulter T660.

Sample	Glucose, mmol/L	MCV, fL			
		Mean[a]	Int. 1	Int. 2	Int. 3
1	5.9	88.3	89.4	87.2	88.3
2	56	90.1	91.1	90.1	89.0
3	84	94.5	96.7	94.6	92.2
4	124	102.0	105.4	101.7	98.8

[a] MCV as reported by the system (mean of the three measuring intervals).

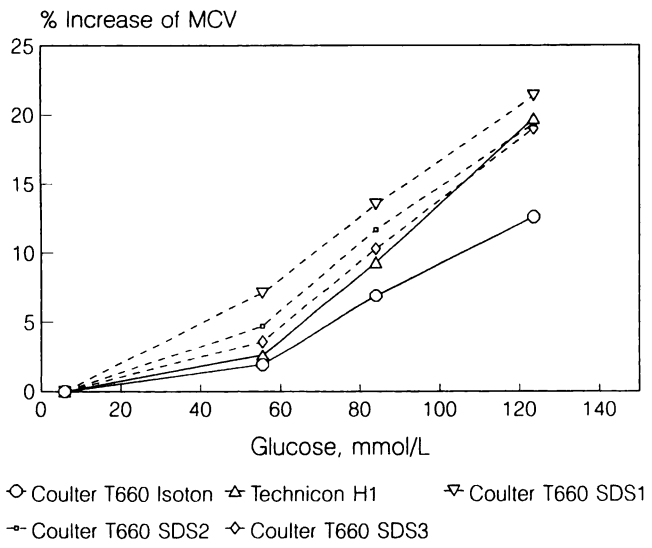

Fig. 4. Influence of SDS on MCV analysis with Coulter T660, reported for three measuring intervals, as compared with Technicon H1.

samples and quality-control samples analyzed with Isoton III-SDS were ~7 fL higher than the results obtained with Isoton III (not shown). Measured variables other than MCV (and calculated hematocrit and MCHC) were independent of the diluent.

Discussion

Here we have shown in vivo as well as in vitro that increased glucose concentrations can result in falsely high MCV results reported by the Technicon H1 and H2 and (to a lesser extent) by the Coulter T660. The effect exceeds between-day imprecision for the Technicon H2 system for glucose concentrations of ~35 mmol/L and higher (based on between-day CV of 0.8%, Testpoint Hematology control, n = 40). These increased glucose concentrations are usually found only in uncontrolled diabetics. Patient S. is an extreme example of this observation.

A possible explanation for this phenomenon was found in the increased osmolality of the plasma (patient S. at admission: 380 mosmol/kg water). Glucose is evenly distributed between erythrocytes and plasma, even without insulin, which allows comparable osmolalities within the erythrocyte and in the plasma. In this way, the cells will retain their normal volume in vivo. The described analyzers dilute the patient's sample. Clearly, the diluent used is not isotonic to the plasma of an individual patient. In case of an increased osmolality because of hyperglycemia, there will be a difference in tonicity between the cytoplasm of the erythrocyte and the diluent used. This will result in rapid swelling of the erythrocytes and therefore an increase of MCV (and decrease of MCHC) during the short time intervals for measurement. At later time points, glucose will exit from the red cells into the large volume of diluent (see below). The hematocrit calculated by use of MCV will be falsely high, but the MCH will be unchanged.

This effect was already reported in 1963 *[4]*. To our knowledge, however, there are no reports on this interference for Technicon H-analyzers (Medline and refs. *[5,6]*). A paper about the measuring principle of Technicon H6000 includes a short statement about a small osmotic effect, which would result in minor changes in erythrocyte volume *[1]*. Experimental conditions and the magnitude of the effect were not described. The current generation-H analyzers are highly different from the H6000 system, so these results are no longer valid for Technicon H1 and H2 analyzers.

Various authors studying this phenomenon in several systems *[7–10]* speculated about the possible cause and differences between systems. Strauchen et al. *[8]* noted that the increase of osmolality is a likely explanation for this finding. Patient H., however, shows us that the osmolality of the plasma can be normal despite hyperglycemia, when the patient is hyponatremic, and patient H. still had increased MCV. This observation is supported by experiments of Beautyman and Bills *[9]*. Acute hyponatremia and hypernatremia produce, respectively, swelling and shrinkage of the erythrocytes in vivo. During analysis, these cells will regain their original (i.e., in normonatremia) volume. In cases of chronic hypo- or hypernatremia the intracellular electrolyte concentrations will change, leading to compensation in vivo, which results in a decrease or increase of cellular volume during analysis. The hyponatremia of patient H. possibly existed for a longer period and may therefore have been (partly) compensated for, such that the increase of MCV caused by the hyperglycemia was limited. However, there are no laboratory results available to support this hypothesis.

The experiments presented in this paper clearly show that incubation time is an important factor in assessing the susceptibility of hematologic analyzers for this interference. Glucose is passively transported across the membrane by a transporter and this process is very fast *[11]*. Directly after contact with the diluent of lower tonicity, the cellular volume will increase rapidly. During the remaining incubation time, glucose is transported out of the cell. Thus, the intracellular glucose concentration will decrease, resulting in a decrease of the initially increased erythrocyte volume. This is clearly shown by the results of the MCV measurements during the three measuring intervals of the Coulter T660 (Table 2); i.e., the MCV decreases in case of longer incubation time. The fact that the incubation time of the Technicon H1 system is longer than the H2 analyzer may explain, at least partly, the observed difference in increase of MCV between the systems. A similar difference in incubation time has been described for Coulter S and Coulter S-plus *[10]*.

The Coulter T660 reported falsely increased MCV results to a lesser degree than did the Technicon H analyzers, so the erythrocytes are less swollen during the analysis by this Coulter system. To find the cause of this difference in susceptibility for hyperglycemic interference, we studied the influence of reagents, the dilution factor, and the incubation time.

Difference in osmolality of the diluents was proposed to be the cause of a similar difference in increase of MCV observed with the Coulter SSR, Ortho ELT-8, and Sysmex CC-800 *[7]*. The osmolality of RBC diluent used by Technicon H1 and H2 was 288 mosmol/kg water, whereas Isoton III diluent contained 322 mosmol/kg water. The difference in osmolality between the plasma and the diluent, which causes swelling of the cells, is indeed lower in the case of Isoton III. However, the system is calibrated with this hypertonic fluid, and the MCV results

reported by the Coulter T660 are corrected for the shrinkage of the red cells in this diluent—which excludes the role of the tonicity of the diluents as an explanation for the difference in increase of cellular volume.

The dilution factor of the sample with the diluent could also contribute to the observed difference in the increase of MCV. This factor is high for both systems (Technicon H1 and H2: 625; Coulter T660: 6250), so the osmolality of the diluted sample will be nearly identical to the osmolality of the diluent. Therefore, an effect of this dilution factor will be unlikely.

The composition of the diluent and particularly the presence of SDS and glutaraldehyde in RBC diluent could also have an influence. Fixing the cells could prevent them from changing the volume because of a decreased function of the glucose transporter and increased rigidity of the cell membrane. This would result in a decreased removal of glucose (and water) from the cell and consequently an impeded change of cellular volume. When we used PBS-SDS in routine operation (instead of RBC diluent), we saw that the presence of glutaraldehyde in RBC diluent could not be the cause of the higher susceptibility of Technicon H1 and H2 analyzers for the false increase of MCV due to hyperglycemia. Kim and Ornstein *[12]* reported that glutaraldehyde would be essential in case no additional protein was added to the diluent, to prevent lysis of red cells. They did not present experimental results to support this finding. Contrastingly, our experiments with the two diluents measured nearly identical red cell counts. This observation rules out the lysis of red cells in the absence of glutaraldehyde.

However, the use of SDS in RBC diluent may account for this observation. Because the assay principle used in Technicon H1 and H2 analyzers requires the presence of SDS, we decided to add SDS to Isoton III in an identical concentration as is used in RBC diluent. The addition of SDS to Isoton III resulted in a general increase of MCV results, probably because the Coulter T660 system was not calibrated with this diluent. This observation is confirmed by previous work of England *[13]*. The percentage increase of MCV due to hyperglycemia is much higher when Isoton III-SDS is used, compared with the use of Isoton III (Fig. 4). Because of the decreasing MCV in time, shown in Fig. 4, we concluded that the use of SDS did not seem to influence the function of the glucose transporter. SDS could, however, affect the rigidity of the cell membrane, or the attachment of the cytoskeleton, by disconnection of bonds between α and β spectrin heterodimers. This could result in a decreased resistance of the membrane to the increased intracellular pressure caused by the uptake of water or, and more likely, inhibit the recovery of normal cell shape and volume.

Differences in incubation time between the systems are also involved. The mean incubation time (incubation time + half of the measuring time) for the three measuring intervals of the Coulter T660 were respectively 12.5, 17, and 21.5 s—the latter approximating the mean incubation time of the Technicon H1 (25 s). This suggests that, if the incubation time of Coulter T660 were to be increased to 25 s and used with Isoton III-SDS, one could expect an increase of MCV similar to that observed with the Technicon H1 (Fig. 4).

Increased urea concentrations to clinically relevant values had no effect (not shown), as has also been observed by Holt et al. *[10]*. Because of the high membrane permeability to urea, the concentration in the cells and in the diluent will probably equilibrate during incubation before measurement.

In conclusion, severe hyperglycemia can result in falsely high MCV results, which will also cause false results for erythrocyte indices calculated with the use of MCV results. Technicon H analyzers appear to be more susceptible to this interference than the Coulter T660. The observed difference between Technicon H analyzers and Coulter T660 in increases of MCV attributable to hyperglycemia appears to be related to the presence of SDS in RBC diluent and to differences in incubation times. We think it worthwhile to be aware of this phenomenon, especially when a Technicon H2 system is used, so as to be able to explain sudden changes in MCV results. Preferably, in cases of severe hyperglycemia, one should be reluctant in reporting MCV, MCHC, and hematocrit results obtained from electronic cell counters. If necessary, a centrifuged hematocrit can be used as a reference and the MCV and additional RBC indices can be calculated. This would prevent unnecessary and costly follow-up tests to find the cause of the spurious macrocytosis.

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