Glucose Determinations in Plasma and Serum: Potential Error Related to Increased Hematocrit

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This study reports the effect of increased hematocrit (packed cell volume) on the rate of decrease in plasma glucose during 6 h in blood specimens from seven newborns (cord blood) and heparin-treated blood specimens from eight adults. Mean hematocrits ranged from 0.43 to 0.75 for the adults and from 0.51 to 0.81 for the infants. The decrease in glucose was related to time and to hematocrit. For example, in adults with a mean hematocrit of 0.43, glucose decreased from 890 ± 40 mg/L to 570 ± 30 mg/L (mean ± SEM) at 6 h, whereas in adults with a mean hematocrit of 0.75, glucose decreased from 890 ± 40 to 200 ± 30 mg/L. In infants with a mean hematocrit of 0.51, glucose decreased from 1010 ± 20 to 480 ± 70 mg/L at 6 h; infants with a mean hematocrit of 0.81 had blood glucose decrease from 1010 ± 20 to 50 ± 30 mg/L. These results indicate that the incidence of hypoglycemia in infants with polycythemia may be overestimated, and emphasize the need for prompt handling of blood samples collected from newborns for glucose determinations.

Additional Keyphrases: pediatric chemistry · newborns · sample handling · glycolysis in blood drawn for glucose determinations · variation, source of

The accuracy of plasma glucose determinations is related to the method of sample collection (1–4); addition of glycolytic inhibitors such as NaF (2–4); separation of serum or plasma from cells; and sample treatment—i.e., cooling specimens on ice or refrigerating them immediately (1) and, particularly, the interval between sampling and assay (1–5). These problems are particularly relevant to glucose determinations in newborn infants. Several investigators (6–8) have demonstrated that glucose consumption is greater in erythrocytes from either premature or full-term infants than in erythrocytes from adults. Because newborns commonly have hematocrits >0.60 and not uncommonly >0.70, we evaluated the effect of hematocrit on results for glucose in blood specimens from newborns and adults as another potential source of laboratory error in glucose determinations.

Methods

Whole blood was obtained from eight healthy adult volunteers and from the umbilical cord of seven normal, full-term infants of appropriate weight for gestational age. Without delay, 4-mL aliquots were treated with sodium heparin (5 int. units/mL of blood), placed in 10 test tubes, and centrifuged (8000 rpm, 5 min). The 10 tubes per specimen were then allocated to five groups. Group 1 was the control and had no further manipulation; the remaining groups had various volumes of plasma removed, resulting in the hematocrit values listed in Table 1. Samples with hematocrits exceeding 0.85 were eliminated from further consideration. Hematocrits were determined in duplicate. In addition, non-heparinized blood from each adult and infant was placed into a tube containing 10 mg of potassium oxalate and 12.5 mg of NaF, and into a tube containing no anticoagulant ( clot tube ). We determined plasma glucose in duplicate 20-μL heparin-treated samples at 0, 1, 2, 4, and 6 h with a glucose analyzer (Model 23A; Yellow Springs Instrument Co., Yellow Springs, OH 45387). Duplicate glucose determinations were made at 1, 2, 4, and 6 h for the NaF-treated specimens and at 2, 4, and 6 h for the clotted specimens. The serum and clot were not separated or agitated during the study. The glucose value at time 0 for the heparin-treated samples for both the infant and adult specimens was used as the reference (time-zero) glucose value for NaF-treated and clotted specimens from infants and adults. All samples were left at room temperature throughout the study (25–27 °C). Results were evaluated by the Student’s t- and paired t-tests.

The study was approved by the Committee of Associates on Human Investigation at the University of South Florida.

Results

Table 1 shows the glucose changes with time for each group. The higher baseline glucose value in infants than in adults partly obscures the significantly more rapid changes in glucose concentrations in infants, both with increasing time and increasing hematocrits.

Decreases in glucose in samples from infants were signifi-
cantly greater than in adults by 2 h at each hematocrit grouping of 0.60 or greater (p <0.05, except for the comparison between 1-h decrements for adult group 5 and infant group 4, where p <0.01). Table 2 shows the effects of anticoagulant on glucose values. There was no significant difference between infants and adults in decrease in glucose in fluoride-treated and clotted samples. In infants, the decrease in glucose in heparin-treated blood specimens (infant group 1) was significantly greater than in fluoride-treated or clotted samples at 6 h (p <0.05). In adults, the decrease in glucose in heparin-treated blood (adult group 1) significantly exceeded that in fluoride-treated samples at 4 h and in fluoride-treated and clotted samples at 6 h (p <0.05).

Discussion

Blood left at room temperature will have a significantly smaller glucose concentration, primarily because of continued erythrocyte glycolysis (1–5). This has led to numerous attempts to find the best method for minimizing this source of error. The promptness with which cells are separated from plasma or serum varies from one laboratory to another. Lin et al. (1) demonstrated the efficacy of cooling specimens on ice. Meites and Saniel-Baney (3) attempted unsuccessfully to prevent any glucose loss for 5 h by using a combination of sodium fluoride and sodium iodoacetate; they decreased the loss of glucose to 11.2% at 5 h, which is comparable with our findings that fluoride-treated samples lost 14–16% of their original glucose. No method has completely eliminated the problem of continued glycolysis. Overfield et al. (4), comparing NaF- and non-NaF-preserved specimens, demonstrated a potential error of >40% in glucose determinations in non-NaF-preserved specimens from adults after 4 h. Meites and Saniel-Baney (3) showed a 67.7% decrement in glucose in heparin-treated samples from newborns during 5 h, as compared with the 55% decrement in 6 h in our specimens from infants (group 1). In contrast, infants with greater hematocrits demonstrated an 18% and 24% decrement in glucose (infant group 3 and 4, respectively) at just 1 h. Delays of 2 h would result in glucose decrements of one-third (infant group 3) to one-half (infant group 4) in infants and one-fifth (adult group 4) to one-fourth (adult group 5) in adults. Infant group 3 (hematocrit 0.71) had an 84% decrease in glucose at 6 h.

For any given hematocrit, specimens from adults contain more cells, because newborns have cells with a larger mean cell volume. This emphasizes even further the more rapid glucose consumption by erythrocytes from infants vs those from adults. The contribution of leukocytes to glucose consumption is probably negligible. Oski and Naiman (6) noted that erythrocyte glucose consumption was only "slightly less" in leukocyte-free preparations than in whole blood. Stjernholm and Manak (9) reported that glucose consumption by leukocytes from adults (0.06 µmol/h per 10^8 cells) was about two to threefold greater than the glucose consumption in adult erythrocytes reported by Oski and Naiman: 268 µmol/h per 10^12 cells, equivalent to 0.0268 µmol/h per 10^8 cells. In our study the erythrocyte:leukocyte ratio was 650:1 for adults and 400:1 for newborns. We found no correlation between leukocyte count and decrease in glucose values. Also, we carefully manipulated blood specimens as little as possible to imitate normal laboratory conditions more closely.

Hematocrit values in newborn infants frequently exceed 0.60 and often exceed 0.70. Higher values have been roughly correlated with hyperviscosity, which is sometimes associated with hypoglycemia. Wirth et al. (10) found 4% of newborn infants had venous-blood hematocrits of 0.65 or greater, and 5% of samples from newborns showed hyperviscosity. Although many infants with hyperviscosity experience symptomatic hypoglycemia, it is possible that any measurement of glucose would be affected by the greater hematocrit; thus the incidence of hypoglycemia in infants with increased packed cell volumes may be overestimated. This further emphasizes the need for prompt handling of blood specimens from such infants, including placing specimens on ice.

We thank Lewis A. Barness for his advice and review of this manuscript.
References


Enzymic Determination of Citrate in Serum and Urine, with Use of the Worthington “Ultrafree” Device

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We describe an enzymic method for conveniently measuring citrate in serum or urine. Interfering enzyme proteins are removed by a disposable ultrafilter (“Ultrafree”; Worthington Diagnostics), obviating the need for hazardous protein precipitants. A 50 mmol/L Tris buffer adequately controls pH, no lactate dehydrogenase is necessary in the reagents, and the reaction of citrate catalyzed by citrate lyase (EC 4.1.3.6) is complete in 2–3 min. Within-run and day-to-day coefficients of variation were 7.5% and 5.4%, respectively. Serum citrate concentrations for 20 apparently healthy persons ranged from 0.08 to 0.17 mmol/L (mean 0.12, SD 0.03). Urinary citrate excretion by six normal volunteers ranged from 2.2 to 4.4 mmol/24 h. We observed no detectable changes in citrate in whole blood stored at room temperature for 90 min or longer. Overall, the method is faster and less hazardous than other methods for citrate that require protein precipitation.

Additional Keyphrases: citrate (pro-3S) lyase - calculous disease - reactions to administration of bank blood - reference interval

The deliberate excess of citrate in stored bank blood can cause symptoms in some recipients, which range from very mild to severe, although studies disagree as to the prevalence of these symptoms (1, 2). The hypocalcemic effect of citrate is believed to be the cause of most of these symptoms, although the concentrations of potassium, phosphate, and lactate (e.g.) may also be important (3). In certain patients, measurement of citrate may be of use in documenting its assumed high concentrations and (or) rate of metabolism.

Studies of inhibition of calcium phosphate precipitation by citrate (4) and of decreased urinary excretion of citrate in persons with recurrent calcium urolithiasis (5, 6) indicate that sufficient urinary citrate may be a factor in prophylaxis of renal stones. Reportedly, administration of bicarbonate to increase urinary citrate may help decrease the recurrence of renal stones (7). These observations could prompt requests for measurement of citrate in serum or urine, or both.

Several specific enzymic techniques have been published (8–13) for measuring citrate by using citrate (pro-3S) lyase (EC 4.1.3.6), which converts citrate to oxaloacetate and acetate. Malate dehydrogenase (EC 1.1.1.37) then converts the oxaloacetate to malate, with NADH consumed in the process. Moellering and Gruber (8) showed that this citrate lyase is highly specific for citrate. However, their preparation of citrate lyase contained a contaminant enzyme, oxaloacetate decarboxylase (EC 4.1.1.3), which required the addition of lactate dehydrogenase (LD; EC 1.1.1.27) to the reagents to offset this potential interference. In most methods published since then, LD is used (6–8, 10). However, Toftegaard-Nielsen (12) described a commercially available citrate lyase preparation that is without such contamination and with which the use of LD is not necessary. He systematically determined optimal reagent concentrations, but the method requires protein precipitation, as have most (8, 10–12), and also requires about 1 h to perform. Others found deproteinization unnecessary, but have either used suboptimal conditions (9) or have not given details of their procedure.

Our modifications allow a more efficient use of reagents and time, combined with the recommended option of using a...