Glyceraldehyde Preserves Glucose Concentrations in Whole Blood Specimens

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Background: Glucose concentrations decrease in blood specimens during transport/processing, primarily because of continuing metabolism (glycolysis) by erythrocytes. Several means to reduce the loss of glucose in blood specimens have been developed, but all have major drawbacks. Glyceraldehyde, which has antiglycolytic activity, was assessed for potential in preserving glucose in blood specimens.

Methods: Heparinized blood from volunteers was treated with glyceraldehyde and other agents. After incubation for various times, plasma concentrations of glucose and other common analytes were determined with prevalent commercial analyzers.

Results: The racemic mixture of glyceraldehyde (D,L-GA) preserved glucose concentrations for up to 8 h at room temperature. Half-maximal effect was attained with 0.9 mmol/L D,L-GA. Trials of the D and L stereoisomers individually indicated that the L isomer (L-GA) was responsible for all or most of the antiglycolytic activity of the racemic mixture. Other related compounds were ineffective. Measurements of most common clinical laboratory analytes were unaffected by the presence of D,L-GA or L-GA.

Conclusions: Glyceraldehyde (D,L-GA or L-GA) effectively preserves glucose concentrations in whole blood specimens for up to 8 h. Specimens collected with D,L-GA or L-GA are suitable for analysis of many analytes commonly comasured with glucose.

Glucose is the loss of glucose from specimens because of glycolysis by erythrocytes during transport and processing (1). In recent years this phenomenon has been more evident as laboratory services have consolidated and many more specimens are transported to distant laboratories for analysis. Several approaches have been proposed to minimize glucose loss, including centrifugation/decantation of plasma immediately after specimen collection (1); refrigeration/cooling on ice during transport (2); addition of antiglycolytic agents such as iodoacetate (3), fluoride (4), or mannose (5) to the collection tubes; and the use of glucose analyzers designed for near-patient testing, at the bedside (6). All of these approaches are in current use, and the use of fluoride in blood collection tubes is prevalent in circumstances where substantial delay between collection and analysis is anticipated; however, all have substantial limitations [for review, see Ref. (7)]. To various degrees, these approaches are limited in efficacy by incomplete inhibition of glycolysis, interference in testing for co-analytes (e.g., electrolytes, creatinine, and urea), disturbance of cellular integrity (e.g., hemolysis), or promotion of leakage of intracellular potassium.

The ideal approach to eliminating glucose loss would provide reasonably stable glucose concentrations for the period needed for transport to a centralized laboratory, avoid costly near-patient analysis, and yield a specimen that was suitable for analysis of many other common analytes so that separate collection of specimens for those analytes was not necessary. From a practical standpoint, the best way to achieve this goal is discovery of an antiglycolytic agent that could be added to collection tubes but did not alter cellular integrity or interfere in common analytical methodologies. Such an agent should also be effective at low concentrations (minimizing volume addition to avoid dilution errors), dissolve rapidly during the collection process, be nontoxic, be stable in the room-temperature storage environment of blood collection devices, and be inexpensive. Here I present studies of the glucose preservation properties of glyceraldehyde, which appears to have potential as an additive in blood collection devices.
Materials and Methods

Subjects
Whole blood specimens collected in tubes containing sodium heparin as anticoagulant were obtained from healthy adult volunteers or from adult diabetic patients visiting an outpatient clinic, after informed consent was obtained. Specimens were used within 1 h of collection. In a few experiments, heparinized whole blood specimens arriving at the clinical laboratory for analysis for glucose and other common analytes were intercepted, and aliquots were removed for experimental purposes. These studies were conducted in accordance with a protocol approved by the Human Studies Committee of Washington University.

Experimental Design
Aliquots of heparinized whole blood (0.96 mL) were pipetted into microcentrifuge tubes containing 0.040 mL of saline or antiglycolytic agent in saline. Antiglycolytic agents were prepared as 250 mmol/L stock solutions within 1 h of use. At various times, plasma was prepared from incubations by centrifugation at 8000 g for 2 min; plasma was decanted into 12 × 75 mm tubes and stored frozen until analysis. All incubations of whole blood were conducted at room temperature (23 °C) on a rotating table shaker, which kept the cells in dispersed suspension. d,l-Glyceraldehyde (d,l-GA), methylglyoxal, d-glyceraldehyde (d-GA), glycolaldehyde, and dihydroxyacetone were obtained from Sigma. l-Glyceraldehyde (l-GA) was obtained from Fisher Scientific.

Analytical Procedures
Glucose analyses were routinely performed on a Cobas MIRA analyzer (Roche Diagnostic Systems), using reagents based on glucose oxidase, manufactured by Sigma Diagnostics. In some experiments, glucose and many other common laboratory analyses were performed on a Vitros 250 Analyzer (Ortho-Clinical Diagnostics), a Hitachi 747 Analyzer (Boehringer-Mannheim) and a RxL Analyzer (Dade Behring).

Statistical Analysis
All results are stated as means ± 1 SD, unless otherwise stated. Correlative studies are reported graphically, after calculation of a least-squares linear regression relationship. Statistical significance was assessed with the Student t-test, with P values <0.05 considered significant.

Results
Stabilization of Blood Glucose Concentrations by d,l-GA
Whole heparinized blood was incubated for 0–8 h in the presence or absence of 10 mmol/L d,l-GA, and the glycolytic loss of glucose was followed over time (Fig. 1). A linear decrease in glucose concentration was evident in incubations without additive, with 38% of the initial glucose concentration lost in 8 h (0.23 mmol/L per hour).

Dose-Dependent Stabilization of Glucose Concentrations by d,l-GA
Whole heparinized blood was incubated for 8 h in the presence of 0–10 mmol/L d,l-GA (Fig. 2). Similar to preceding experiments, glucose concentrations declined...
from 4.8 ± 0.1 mmol/L to 2.6 ± 0.2 mmol/L (46% decline) when no d,l-GA was present, but increasing concentrations of d,l-GA effectively preserved glucose concentrations so that at 5 mmol/L d,l-GA, decreases in glucose were nearly completely eliminated (residual glucose, 4.6 ± 0.3 mmol/L at 8 h). The concentration of d,l-GA that reduced glycolytic loss by one-half was ~0.9 mmol/L. The presence of d,l-GA did not appear to interfere in the glucose assay at any of the concentrations examined (Fig. 2).

Efficacy of d,l-GA in Patient Specimens

Specimens arriving at the clinical laboratory for glucose and other common laboratory analyses were split into three aliquots: one was analyzed immediately to establish the initial glucose concentration in each specimen; one was incubated for 8 h without additive; and the third aliquot was incubated for 8 h in the presence of 10 mmol/L d,l-GA. Results are presented by plotting initial glucose concentration against glucose concentrations in either the presence or absence of d,l-GA (Fig. 3). The line generated with incubations without additive had a slope of 1.01 and an intercept of ~2.2 mmol/L, indicating that 8 h of incubation reduced glucose concentrations an average of 2.2 mmol/L regardless of starting concentration (0.28 mmol/L per hour). The relationship of initial glucose to glucose concentration after 8 h of incubation in the presence of d,l-GA also yielded a slope of 1.01, but the intercept was only 0.3 mmol/L, indicating that d,l-GA reduced the extent of glucose loss more than sevenfold compared with the absence of d,l-GA. Furthermore, d,l-GA appeared to be equally effective regardless of the starting glucose concentration, which ranged from 3.9 to 13.4 mmol/L.

**Table 1. Glucose preservation activity of compounds related to d,l-GA.**

<table>
<thead>
<tr>
<th>Agent</th>
<th>At 0 h</th>
<th>At 8 h</th>
<th>Decrease, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additions</td>
<td>4.8 (0.3)</td>
<td>2.8 (0.3)</td>
<td>41</td>
</tr>
<tr>
<td>d,l-GA</td>
<td>4.7 (0.2)</td>
<td>4.8 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td>d-GA</td>
<td>4.7 (0.1)</td>
<td>3.9 (0.1)</td>
<td>17</td>
</tr>
<tr>
<td>L-GA</td>
<td>4.8 (0.1)</td>
<td>4.8 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td>Methylglyoxal</td>
<td>4.5 (1.0)</td>
<td>2.6 (0.2)</td>
<td>43</td>
</tr>
<tr>
<td>Glyceric acid</td>
<td>4.7 (0.1)</td>
<td>2.7 (0.2)</td>
<td>42</td>
</tr>
<tr>
<td>Dihydroxyacetone</td>
<td>4.5 (0.1)</td>
<td>2.4 (0.1)</td>
<td>46</td>
</tr>
<tr>
<td>Glycolaldehyde</td>
<td>4.8 (0.1)</td>
<td>2.3 (0.1)</td>
<td>52</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>5.0 (0.1)</td>
<td>3.2 (0)</td>
<td>38</td>
</tr>
</tbody>
</table>

* All compounds were present at a final concentration of 10 mmol/L.
* SD in parentheses.

**Effect of d,l-GA on Erythrocyte Potassium and Lactate Homeostasis**

Some antiglycolytic measures, such as storage on ice, so effectively inhibit erythrocyte metabolism that the cells are unable to maintain the potassium gradient between plasma and intracellular fluid, leading to significant increases in plasma potassium concentrations. The time course of potassium concentration changes was followed for 8 h in whole blood incubations under three conditions: (a) incubation at room temperature (without additions); (b) incubation at room temperature with 10 mmol/L d,l-GA; and (c) incubation at 4 °C (without additions). In room temperature incubations without additive, potassium decreased modestly from 4.0 ± 0 mmol/L at 0 h to 3.5 ± 0.1 mmol/L at 8 h. Incubation for 8 h in the presence of d,l-GA produced an increase of similar magnitude (to 4.5 ± 0 mmol/L). In contrast, incubation at 4 °C, which has been advocated as an effective way to prevent glucose loss for short periods (2), produced an increase to 6.0 ± 0.1 mmol/L.

An analogous experiment examining the time course of lactate concentration changes found that the time-dependent increase in plasma lactic acid was not prevented by d,l-GA, whereas incubation on ice preserved initial lactate concentrations (data not shown).

**Glucose Preservation Activity of Related Compounds**

Several compounds related biochemically or structurally to d,l-GA were tested for their ability to preserve specimen glucose; incubations without inhibitor and with d,l-GA were included for comparison (Table 1). All compounds were tested at a final concentration of 10 mmol/L, and incubations were conducted at 0 and 8 h for each compound. Only the d and l stereoisomers of glyceraldehyde (d-GA and l-GA) prevented glucose loss. The inhibition by d-GA was only partly effective, reducing glucose loss from 41% (no additions) to 17%. Loss of glucose was completely prevented by l-GA. The partial inhibition by d-GA could have been attributable to small
contamination of the d-GA preparation with the l isomer, and the efficacy of d,l-GA could be attributable entirely to the action of the l isomer in this preparation. To test this possibility, an experiment varying the concentration of l-GA was performed in analogy with the earlier experiment varying the d,l-GA concentration (see above). If l-GA is the active portion of the d,l-GA mixture (and d-GA is inert), then the concentration of l-GA needed to inhibit glycolysis will be one-half that of d,l-GA. The concentration that reduced glucose loss by 50% was 0.65 mmol/L, which was close to one-half of the d,l-GA concentration needed (see above). Furthermore, 2.5 mmol/L l-GA was nearly completely effective in eliminating glycolytic loss during an 8-h incubation (data not shown), whereas twice that concentration of d,l-GA was needed for comparable influence. These results were interpreted to suggest that the antiglycolytic action of d,l-GA was attributable to the l-GA component of the racemic mixture.

**EFFECT OF D,L-GA AND L-GA ON COMMON CLINICAL CHEMICAL TESTS**

The potential for d,l-GA and l-GA to interfere in testing for commonly ordered co-analytes was investigated by drawing blood from six diabetic patients in a clinic setting and adding to aliquots 10 mmol/L d,l-GA, 5 mmol/L l-GA, or the same volume of saline. The aliquots were centrifuged immediately, and plasma was decanted for analysis on three common laboratory automated systems: Vitros 250 (Ortho-Clinical Diagnostics), RxL (Dade Behring), and Hitachi 747 analyzers. The analytes tested were those in the standard "basic metabolic panel/comprehensive metabolic panel" (Table 2). Glucose results were similar on all three analyzers, which suggested that l-GA and d,l-GA do not effect either glucose oxidase methods (Vitros 250 and Hitachi 747) or methods based on hexokinase (RxL). The other analytes examined were also free of significant interference from either l-GA or d,l-GA, except creatinine. Creatinine analyses on the Vitros 250 and Hitachi 747 (both enzymatic methods) were not influenced by the presence of either d,l-GA or l-GA, but the alkaline picrate method of the Dade Behring RxL was highly susceptible to positive interference by either form of GA (Table 2). Aspartate aminotransferase results were modestly depressed in the presence of GA on all three analyzers, with greater decreases observed on the Dade Behring RxL and lesser effects in specimens treated with l-GA, but these decreases were too small to reach statistical significance (Table 2).

**Table 2. Effect of D,L-GA or L-GA on glucose and other analytes, measured on three analyzers."**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>NoAdda</th>
<th>d,L-GA</th>
<th>LGAb</th>
<th>NoAdda</th>
<th>d,L-GA</th>
<th>LGAb</th>
<th>NoAdda</th>
<th>d,L-GA</th>
<th>LGAb</th>
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<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>11.0 (6.4)</td>
<td>10.9 (6.5)</td>
<td>10.9 (6.5)</td>
<td>10.5 (6.2)</td>
<td>10.5 (6.2)</td>
<td>10.6 (6.3)</td>
<td>10.6 (6.1)</td>
<td>10.5 (6.0)</td>
<td>10.5 (5.9)</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>142 (2)</td>
<td>142 (1)</td>
<td>143 (1)</td>
<td>140 (2)</td>
<td>140 (2)</td>
<td>140 (2)</td>
<td>139 (2)</td>
<td>139 (2)</td>
<td>139 (2)</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.5 (1.1)</td>
<td>4.6 (1.2)</td>
<td>4.6 (1.1)</td>
<td>4.5 (1.1)</td>
<td>4.5 (1.2)</td>
<td>4.5 (1.1)</td>
<td>4.6 (1.0)</td>
<td>4.6 (1.1)</td>
<td>4.6 (1.1)</td>
</tr>
<tr>
<td>Chloride, mmol/L</td>
<td>110 (4)</td>
<td>110 (5)</td>
<td>109 (4)</td>
<td>105 (4)</td>
<td>105 (4)</td>
<td>105 (3)</td>
<td>105 (3)</td>
<td>105 (3)</td>
<td>105 (3)</td>
</tr>
<tr>
<td>Total CO₂, mmol/L</td>
<td>26 (3)</td>
<td>25 (4)</td>
<td>25 (4)</td>
<td>25 (3)</td>
<td>24 (2)</td>
<td>24 (3)</td>
<td>26 (3)</td>
<td>25 (3)</td>
<td>26 (3)</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>80 (18)</td>
<td>71 (18)</td>
<td>80 (18)</td>
<td>88 (18)</td>
<td>327 (35)c</td>
<td>194 (27)c</td>
<td>97 (18)</td>
<td>80 (9)</td>
<td>88 (18)</td>
</tr>
<tr>
<td>Urea, µmol/L</td>
<td>6.4 (2.8)</td>
<td>6.4 (2.8)</td>
<td>6.4 (2.8)</td>
<td>5.4 (2.5)</td>
<td>5.4 (2.5)</td>
<td>5.4 (2.5)</td>
<td>5.7 (2.5)</td>
<td>5.7 (2.5)</td>
<td>5.7 (2.5)</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>2.1 (0.1)</td>
<td>2.1 (0.1)</td>
<td>2.2 (0.1)</td>
<td>2.1 (0.1)</td>
<td>2.2 (0.1)</td>
<td>2.2 (0.1)</td>
<td>2.1 (0.1)</td>
<td>2.1 (0.1)</td>
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<tr>
<td>Bilirubin, µmol/L</td>
<td>15.4 (10.3)</td>
<td>15.4 (10.3)</td>
<td>15.4 (10.3)</td>
<td>6.8 (3.4)</td>
<td>8.6 (1.7)</td>
<td>6.8 (1.7)</td>
<td>5.1 (1.7)</td>
<td>6.8 (3.4)</td>
<td>6.8 (3.4)</td>
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<tr>
<td>Albumin, g/L</td>
<td>38 (2)</td>
<td>38 (2)</td>
<td>38 (2)</td>
<td>37 (3)</td>
<td>37 (3)</td>
<td>37 (3)</td>
<td>37 (2)</td>
<td>36 (2)</td>
<td>37 (2)</td>
</tr>
<tr>
<td>Protein, g/L</td>
<td>67 (3)</td>
<td>65 (4)</td>
<td>66 (4)</td>
<td>65 (2)</td>
<td>65 (3)</td>
<td>66 (2)</td>
<td>64 (2)</td>
<td>63 (3)</td>
<td>63 (3)</td>
</tr>
<tr>
<td>Alk Phos,c U/L</td>
<td>76 (19)</td>
<td>76 (19)</td>
<td>77 (19)</td>
<td>78 (12)</td>
<td>76 (14)</td>
<td>76 (14)</td>
<td>85 (6)</td>
<td>83 (7)</td>
<td>84 (7)</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>37 (18)</td>
<td>33 (14)</td>
<td>35 (15)</td>
<td>31 (22)</td>
<td>23 (14)</td>
<td>26 (15)</td>
<td>32 (21)</td>
<td>25 (16)</td>
<td>29 (20)</td>
</tr>
</tbody>
</table>

a All results are expressed as means with SD in parentheses.
b NoAdd, no additive; Alk Phos, alkaline phosphatase; AST, aspartate aminotransferase.
c P < 0.001.

**Discussion**

Considerable effort has been expended in the past to find a highly effective preservative of glucose for blood collection that does not interfere in other common clinical
chemical tests, does not cause hemolysis or other loss of cellular integrity, is nontoxic, is stable for storage at room temperature, and is inexpensive. GA, and specifically l-GA, seems to meet these criteria better than any currently available alternative. Specimens containing GA were suitable for most common clinical chemical determinations, although careful evaluation will be needed in individual laboratories to determine the potential for interference in such methodologies as the alkaline picrate method for creatinine. Unlike the most prevalently used glucose preservation agent, fluoride ion, GA does not cause hemolysis, and potassium concentrations in plasma from GA-treated specimens remain suitable for assessment of potassium homeostasis for up to 8 h. From the standpoint of stability and solubility, GA seems ideal as an additive because it is highly soluble (30 g/L) and, according to the source, Sigma Chemical, d,l-GA can be stored at room temperature in the crystalline form. Because l-GA is fully effective at concentrations as low as 2.5 mmol/L, the actual amount of l-GA needed for the standard evacuated 7-mL collection tube is 1.58 mg. This small dose limits the expense of use of GA and eliminates the potential that volume dilution of the specimen by the additive might decrease glucose and other determinations, in contrast to the equivalent fluoride/oxalate 7-mL tube, which contains >31 mg of additive. Volume dilution of specimen by additive becomes more important when collection tubes are only partially filled.

Prevalent approaches to minimizing glycolytic loss of glucose have considerable limitations. The use of fluoride or iodoacetate to inhibit cellular glycolytic enzymes is only partially effective; although significantly decreased, glycolysis continues in the presence of either agent, particularly in the first few hours after collection (9, 10). Iodoacetate interferes in at least some methods of glucose analysis (11). Specimens collected in standard fluoride/oxalate tubes are invariably hemolyzed, which makes them unsuitable for analysis of other important analytes that are frequently requested in conjunction with glucose, such as potassium. The use of mannose as an antiglycolytic agent has been hampered by reports of interference of mannose in several glucose methods (12, 13). Mannose was also judged unsuitable for preservation of specimens for electrolyte analysis because of appearance of cellular potassium in the plasma phase during storage (14). Immediate cooling of specimens and transportation on ice effectively preserves glucose concentrations (2), but it imposes additional costs and burden on the transportation process. Because metabolism is reduced by cooling, cellular potassium rapidly diffuses into the plasma phase of whole blood specimens, which significantly increased plasma potassium concentrations after 1 h (2). The use of near-patient analytical devices to measure glucose immediately after specimen collection effectively eliminates glucose loss, but these analytical devices typically are expensive to operate and place a considerable burden on patient care staff. Glucose meters are a prevalent and relatively inexpensive example of this approach, but they offer only glucose determinations (7).

The antiglycolytic effect of l-GA has been noted previously, but l-GA has never been tested as an additive for preservation of blood specimens for glucose analysis [for review, see Ref. (15)]. The ability of l-GA to inhibit the formation of glucose from d-GA in slices of rat kidney cortex-mix was documented in 1966 (16). Thornalley and Stern (17) noted l-GA inhibition of lactate/pyruvate formation from radioactive glucose in erythrocytes but did not measure glucose concentrations in blood or plasma in their experiments. Glyceraldehyde has been investigated as an agent to promote insulin secretion from isolated pancreatic islets (15); both d-GA and l-GA were effective in promoting secretion. The action of d- and l-GA was related to autooxidation to methylglyoxal, which is a highly reactive inhibitor of many cellular functions.

The mechanism of l-GA inhibition of glycolysis in erythrocytes has not been fully established. l-GA is poorly metabolized (15, 16), through conversion to glyceraldehyde (17). Hexokinase has been proposed as the site of inhibition by l-GA (17), through condensation with dihydroxyacetone phosphate to form sorbose-1-phosphate, which is an inhibitor of hexokinase (18). On the basis of this hypothesis, I anticipated that l-sorbose might mimic the antiglycolytic activity of l-GA, through cellular conversion to sorbose-1-phosphate, but sorbose was not effective at concentrations similar to those of d,l-GA that were effective in preserving glucose concentrations. It is also possible that l-GA inhibits glycolysis through autooxidation to methylglyoxal or pyruvaldehyde, which are known to form covalent adducts with proteins and to inhibit many cellular processes (15). However, results from my study argue against this mechanism. Both d- and l-GA readily autooxidize, but only l-GA seemed to be the active agent in my experiments. Methylglyoxal and related compounds were ineffective as glucose preservation agents when incubated with erythrocytes at the same concentration that was effective for l-GA. Further studies are needed to determine the metabolic fate of l-GA in erythrocytes and to determine the site of inhibition of glycolysis by l-GA or a metabolic product.

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References


2. Lin YL, Smith CH, Dietzler DN. Stabilization of blood glucose by


