The Coulometric Determination of Glucose in Human Serum

Robert K. Simon,* Gary D. Christian,† and William C. Purdy

The coulometric titration method is combined with the use of an enzymatic analytic reagent for the determination of glucose in human serum. The glucose in 25 μl of serum is determined in a protein-free filtrate (PFF) with an accuracy of ± 3% and a coefficient of variation of approximately 2%. The procedure routinely covers a concentration range of 25–250 mg/100 ml. Calibrations are linear to at least 450 mg./100 ml. with zero intercept. Glucose oxidase specifically catalyzes the aerobic oxidation of glucose to hydrogen peroxide. The peroxide reacts with iodide, in the presence of molybdenum (VI) catalyst, to form iodine. A known excess of thiosulfate reduces the iodine as it is produced. The reagents and the sample are incubated at 25.0° and pH 5.1. After 15 min., the pH is adjusted to 8.0 with phosphate reagent to stop the enzymatic reaction. The residual thiosulfate is titrated coulometrically with iodine at pH 8.0 to a dead-stop end point at a generating current of 0.4825 mA. The difference between the sample and thiosulfate reagent titers is proportional to the glucose concentration. The method is empirical. Peroxide-reducing impurities in the glucose oxidase preparation and mutarotation equilibrium prevent the complete recovery of glucose under the conditions of the experiment. Calibrations are reproducible from day to day and week to week. Reagents and the PFF constitute a negligible titration blank. Only 1 calculation is necessary. A simplified apparatus and procedure for the preparation of PFF’s permits 15 manual determinations per hour. Coulometric assays of commercial serum controls are accurate to within 3–4%. Data indicate that the precision of the coulometric method exceeds that of the AutoAnalyzer, Folin-Wu, Glucostat, and Nelson-Somogyi procedures. The proposed method is free from interferences at normal serum levels.

The determination of glucose in biologic samples has been a subject of intense research for over a century. Henry (7) has reviewed in detail the commonly used quantitative glucose procedures. These include the

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copper oxidizing agents, (Folin-Wu, Benedict, Nelson-Somogyi) and ferricyanide (Folin-Mamros, Halgedorn-Jensen), as well as the anthrone method. More recent studies have centered on the specific aerobic oxidation of glucose by glucose oxidase (E.C.1.1.3.4, beta-D-glucose:oxygen oxidoreductase). Although 2-deoxy-D-glucose was found to be the only carbohydrate interference (one-tenth as sensitive as glucose), several authors (6, 7) have reported that colorimetric enzymatic procedures were sensitive to added preservatives and nonglucose substances (uric acid, bilirubin, glutathione). Some work has been reported in which the hydrogen peroxide produced is determined by a direct chemical reaction (10–12), thus alleviating some interferences found in methods employing a second enzymatic step to produce a chromogen from hydrogen peroxide.

Christian (2) has shown that the indirect coulometric determination of microgram quantities of hydrogen peroxide is precise and accurate. Based on this observation, a coulometric glucose assay was designed utilizing the aerobic oxidation of glucose to form hydrogen peroxide.

Experimental

Apparatus

Constant-current coulometric titrations are performed with a Chris Feld Microcoulometric Quantalyzer or the equivalent. The titration cell is similar to the one previously described (5). The titration end point is detected biamperometrically by impression of 200 mv between 2 platinum-foil electrodes with a Sargent Polarograph, Model XV. The amperometric current is recorded on the polarograph (3). A dead-stop technic is performed as follows: with the electrodes immersed in the solution, the pen of the polarograph (at a sensitivity of 0.01 μA/mm.) is set at the 50-mm. mark by the displacement knob. The titration is continued until the indicator current (pen) reaches the 100-mm. deflection point (0.5 μa indicator current). The indicator current remains small until the end point is reached.

Figure 1 illustrates the apparatus used to prepare a PFF from serum for the coulometric method. The apparatus is designed around a Millipore filter holder (Millipore Filter Corp., Bedford, Mass.), 1 × 6 in., with a 25-mm. coarse-grade frit. Whatman fiber-glass filters (25 mm.) are used to retain the precipitate.

Reagents

All reagents are prepared with reagent-grade chemicals and distilled water. pH measurements were made at 25° with a Leeds and Northrup pH meter. Spectrophotometric measurements were made with a Beckman Model DB spectrophotometer.
Barium hydroxide  A 1.8% (w/v) solution is prepared by dissolving 18.0 gm. of Baker's \( \text{Ba(OH)}_2 \) in 1 L of water, filtering off any carbonate residue, and storing in a polyethylene bottle fitted with a \( \text{CO}_2 \) trap. A 20-ml. aliquot of the zinc solution is slowly titrated (approximately 2 ml./min.) with the barium reagent to a phenolphthalein end point. The zinc solution is diluted with water—to be equivalent to the barium reagent within ± 0.05 ml.

Composite reagent  A 2.84-gm. portion of ammonium molybdate, \( (\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O} \); 3.70 ml. of sodium thiosulfate, \( \text{Na}_2\text{S}_2\text{O}_3 \) (0.1 N);* and 41.5 gm. of potassium iodide, KI, are dissolved and diluted to 950 ml. with boiled water. The pH is adjusted to 7.0 with anhydrous sodium carbonate, \( \text{Na}_2\text{CO}_3 \) (approximately 1.17 gm.), and the entire solution is diluted to 1 L. The equivalent concentrations of this reagent

*Prepared as described by Kolthoff and Sandell (9), but not standardized. The thiosulfate is standardized in the titer step.
are: \( \text{Mo(VI)} \), \( 1.25 \times 10^{-3} \text{ M} \); \( \text{Na}_3\text{S}_2\text{O}_3 \), \( 3.75 \times 10^{-4} \text{ M} \) (0.375 \( \mu \text{Eq./ml.} \)); and 0.25 M KI. A 2-ml. aliquot of this reagent gives a 150-sec. titration titer at 482.5 \( \mu \text{A} \). Using 25 \( \mu \text{L} \) of serum, the thiosulfate is sufficient to reduce iodine equivalent to that amount formed from 300 mg. of glucose per 100 ml. Air oxidation of iodide is not detected for 1 week. After this time a small decrease in the titer occurs at the rate of about 0.05 \( \mu \text{Eq./ml./week} \). A stock thiosulfate solution (0.1 N) may be added in the calculated amount to restore the initial titer without significantly changing other reagent conditions.

**Glucose oxidase** A 250-mg. portion of glucose oxidase powder (30 units/mg., Nutritional Biochemicals Corp.) is triturated with 0.6 M sodium acetate buffer, pH 5.1 (pH adjusted with acetic acid), in a glass mortar and diluted to 100 ml. with buffer. This reagent, when stored at 0\(^\circ\), can be used for 1 week without loss in activity that significantly affects glucose recoveries.

**Sodium phosphate** A 0.43 \( \pm \) 0.02 M solution of disodium phosphate is prepared by dissolving 115.3 gm. of \( \text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O} \) in water and diluting to 1 L. An equivalent amount of anhydrous salt can be used.

**Zinc reagent** A 2\% (w/v) solution is prepared by dissolving 20.0 gm. of \( \text{ZnSO}_4 \) in water and diluting to 1 L.

**Glucose standards** A stock solution (450 mg./100 ml.) is prepared by dissolving 0.4500 \( \pm \) 0.0001 gm. of Baker’s Analyzed Dextrose (dried 1 hr. at 105\(^\circ\)) in water and diluting to 100 ml. Appropriate standards between 2.5 and 50.0 \( \mu \text{Eq./ml.} \) are prepared by dilution of the stock solution.

**Procedures**

Glucose is converted to hydrogen peroxide, which oxidizes iodide to iodine. The iodine reacts with an equivalent amount of thiosulfate as it is produced. The excess thiosulfate remaining (known amount taken) is titrated with electrogenerated iodine.

\[
\text{C}_6\text{H}_12\text{O}_6 + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{glucose oxidase}} \text{C}_6\text{H}_12\text{O}_7 + \text{H}_2\text{O}_2 \quad (1)
\]

\[
\text{H}_2\text{O}_2 + 2\text{I}^- + 2\text{H}^+ \xrightarrow{\text{Mo(VI)}} 2\text{H}_2\text{O} + \text{I}_2 \quad (2)
\]

\[
\text{I}_2 + 2\text{S}_2\text{O}_3^{2-} \rightarrow 2\text{I}^- + \text{S}_4\text{O}_6^{2-} \quad (3)
\]

**Coulometric Deproteinization**

PFF reagents—\( \text{Ba(OH)}_2 \) and \( \text{ZnSO}_4 \)—are added with 1-ml. tuberculin syringes. Serum samples are added with a 50-\( \mu \text{L} \). Hamilton precision
microsyringe. Syringes give adequate mixing of serum and reagents.

To the pyrex reservoir of the filtration apparatus, add in order: 0.50 ml. of barium reagent, a 25 µl. serum sample (rinse the syringe once with the added barium reagent), and 0.50 ml. of zinc reagent. Immediately, open the stopcock, apply vacuum, and filter off the BaSO₄-protein precipitate. Close the stopcock, add about 1 ml. of distilled water to the reservoir, open the stopcock, and apply vacuum. The filtrate is collected in the 50-ml. titration cell. Successive samples are manually run every 2-3 min.

**Measurement**

Add 2.0 ml. of composite reagent to the filtrate in the cell. At zero time add 2.0 ml. of enzyme reagent. Incubate the solution at 25.0 ± 0.1°. At 15.0 ± 0.5 min., add 10.0 ± 0.05 ml. of phosphate reagent. Titrate the excess thiosulfate with generated iodine at a generating current of 482.5 μA (for 25-µl. sample) and a polarograph sensitivity of 0.01 μA/mm. The titration time is called the titer excess. An aqueous standard equivalent to 100 mg. glucose per 100 ml. is routinely run without deproteinization.

**Titer**

The composite, enzyme, and phosphate reagents are titrated together under the same conditions as above, without a 15-min. incubation period or deproteinization. The titration value obtained is called the titer.

**Calculation**

The concentration of glucose in serum is calculated as follows:

\[
\text{Sample time (sec.)} = \text{titer (sec.)} - \text{titer excess (sec.)}
\]

(4)

\[
\frac{\text{Standard sample time (sec.)}}{\text{Serum sample time (sec.)}} \times 100 \text{ mg.} / 100 \text{ ml.} = \text{mg. glucose per 100 ml. serum}
\]

(5)

**Preliminary Studies**

**Mutarotation and Inhibitors**

Adams et al. (1) reported that the rate of catalysis of glucose oxidation by glucose oxidase depends on the mutarotation of alpha-glucose to beta-glucose. Their data showed that plots of per cent glucose recovered and per cent beta-glucose vs. time were identical. The authors have recovered in 15 min., 82 and 62% of added glucose at 35° and 25°, respectively, with 150 units of Nutritional Biochemicals glucose oxidase. Ninety per cent glucose was recovered in 1 hr. using Sigma “purified” enzyme at 25°. Although the mutarotation-temperature dependence was not measured, these data seem to indicate that the
increased yield at higher temperatures may be due to more of the glucose being in the beta form. An increase in temperature is known to shift the glucose equilibrium to the beta form. Increased temperature and long incubation times are not recommended for routine analysis because of danger of air oxidation of iodide. In addition to the dependence of total glucose recovery on mutarotation, several workers have reported that substances present in commercial glucose oxidase attack peroxide. In our laboratory, incubation of standard peroxide solutions with commercial glucose oxidase caused a measurable decrease in the peroxide concentration (subtracting for self-decomposition of peroxide). The per cent recovery of peroxide after a 15-min. incubation with glucose oxidase solution at 25° was 35.4%. However, if the glucose oxidase solution was boiled for 15 min. prior to the 15-min. incubation with peroxide, the recovery of hydrogen peroxide was 93.4%. Brief atomic absorption spectrophotometric studies on the digested enzyme preparation indicated that the glucose oxidase contained 8 ppm of zinc and 12 ppm of calcium.

Some commercial suppliers list the catalase content of glucose oxidase since catalase decomposes peroxide. Pardue (12) showed that sodium azide, a known catalase inhibitor, did not alter the rate of the glucose oxidase reaction. The authors tested sodium azide, 2,4-dichlorophenol, and cyanide (catalase inhibitors) and found no effect of these reagents on the absolute recovery of glucose by the coulometric method. These data would suggest that impurities other than catalase are present, although it has not been definitely demonstrated that catalase is inhibited by these chemicals in our media. Pure glucose oxidase, supplied by Sigma and several other companies, gave a slight peroxide effect; only 1.5% of a hydrogen peroxide standard was lost after 1 hr. (in the presence of iodide and thiosulfate ions). The latter preparations, however, are too expensive for use in routine glucose determinations. Mutarotation and peroxide-active substances present in glucose oxidase thus limit the recovery of glucose. In the coulometric method, reaction conditions were empirically selected to yield recoveries which were sufficient for analytically reproducible results.

Most glucose methods in the literature are based on incomplete recovery of glucose. This factor is usually overcome by comparing the standard and the sample under identical conditions. Sunderman and Sunderman (15) are the only workers to actually report absolute recovery values for their method. The proposed coulometric procedure, therefore, does not differ from those in the literature in this respect. Mutarotation and enzyme (peroxide-reducing) impurities appear to be the main factors limiting glucose recovery in this procedure.
Glucose Oxidase

The effect of enzyme concentration on the recovery of glucose was studied. Figure 2A represents a plot of the percent recovery of glucose* vs. the units of glucose oxidase added. (One enzyme unit will catalyze

*One mole of glucose is converted to 1 mole of H₂O₆ (2 equivalents) which in turn is equal to 2 equivalents in the titration (see Equations 1, 2, and 3). % Recovery of glucose = (\(\mu\text{Eq. } \text{S}_4\text{O}_6\text{O}^\text{−}\text{ consumed per microequivalent of glucose taken}\) \(\times 100.\)
Sigma Chemical Co. and Nutritional Biochemicals Corp., were compared as received. A total of 90 \( \mu \text{g} \) of glucose standard were incubated with 150 units of each enzyme. The recoveries after 15 min. were 77 ± 2 and 62 ± 3% for several different lots of the Sigma and Nutritional glucose oxidase, respectively. The data indicated a significant difference between the 2 enzyme preparations, but not between different lots of the same enzyme. An important conclusion was that the same commercial enzyme preparation must be used during a given series of glucose determinations, or else different manufacturer's preparations must be individually calibrated. Unfortunately, after preliminary work the Sigma brand was not available owing to manufacturing difficulties. Thus, the Nutritional Biochemicals enzyme was used. However, the authors would recommend the Sigma preparation because of the higher recovery of glucose.

**Effect of Incubation Time**

Figure 2B is a plot of the per cent glucose recovery vs. incubation time for 250 units of glucose oxidase added per sample. The recovery of glucose rapidly increased during the first 15 min. and thereafter leveled off sharply. For 250 units of added enzyme, a recovery of about 62% was observed after 15 min. The 15-min. incubation point was in the area of greater slope than longer incubation times (greater than 20 min.). However, the enzymatic reaction was stopped within 10–15 sec. by adjustment to pH 8.0. Thus, the choice of a 15-min. incubation period was more economical when considering the time element and introduced an uncertainty of less than 0.2%. Larger amounts of enzyme (greater than 400 units) generally gave smaller percentage recoveries. Thus, the reduction of peroxide by enzymatic impurities appeared to be dependent on concentration and time. There appeared to be a competition for peroxide by the iodide reaction and the enzymatic impurity. Below 400 units of enzyme, this competition was favorable enough (to the iodide reaction) to yield recoveries (62%) adequate for analytic measurement of glucose. In addition, the recovery was shown to be reproducible (Table 1).

**Thiosulfate Addition**

If thiosulfate was added after the 15-min. incubation, the glucose recovery was 10–11% (relative) lower than if added prior to the enzyme reagent. This probably indicated the loss of iodine by volatilization and/or reaction with the enzyme in the absence of thiosulfate. Proteins, including glucose oxidase, have been shown to react with oxidizing agents (4). Titration of thiosulfate before and after 15-min. incubations...
Table 1. Coulometric Calibration*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose found (mg./100 ml.)</th>
<th>Glucose taken (mg./100 ml.)</th>
<th>Recovery (%)†</th>
<th>Coeff. of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Standard</td>
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<td></td>
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<tr>
<td>Serum</td>
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<td>Serum</td>
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<tr>
<td>Serum</td>
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</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Glucose found (mg./100 ml.):

- Serum 100 3 — 69.2 1.3
- Serum 50 3 — 72.1 3.7
- Serum 50 3 — 77.1 0.29
- Versatol-A 50 3 — 79.1 1.2
- Serum 50 3 — 83.5 1.5
- Serum 100 3 — 85.6 2.7
- Versatol-A 50 3 198.5 197.0 1.5
- Versatol-A 50 3 300.0 302.7 0.94
- Total 50

* Calibrations were run on Days 1, 3, and 6 over a one-week period. Generation currents of 0.4825 and 0.9650 ma were used for 25 and 50- to 100-μl samples, respectively.
† See footnote, p. 469.
‡ See Equation 5.

Effects of Reagents on Titer

The effect of the reagents on the titration titer was studied. The components of the composite reagent, glucose, and the deproteinization reagents had no effect. The enzyme and phosphate reagents gave small titration blanks (approximately 3% relative). Titration of enzyme and all other reagents after a 15-min. incubation gave the same result. The 3% increase was reproducible. Therefore, it was necessary to repeat precisely the addition of enzyme and phosphate reagents from sample to sample. Since glucose standards and samples were titrated under identical conditions, the small enzyme-phosphate blank canceled out.

Effect of Oxygen Concentration, pH, and Temperature

Pardue and Simon (13) studied the effects of oxygen concentration, pH, and temperature on the enzymatic reaction. Electroanalytic reaction rate and automated titrimetric methods were used. These authors reported that the dissolved oxygen concentration in a well-stirred
aqueous solution was sufficient to support the oxidation of 0.50 M glucose by glucose oxidase at the same rate for at least 30 min. pH and temperature effects were studied by titrimetric pH-stat and automated amperometric assay procedures. The observed pH maximum in acetate buffer was 5.0–5.2. The enzymatic reaction was carried out at pH 5.1 in the coulometric method. The final buffer (acetate) concentration of at least 0.1 M was necessary to yield reproducible recovery of glucose. The final acetate concentration (in the incubation mixture) was 0.2 M. The enzymatic reaction rate (13) was reported to increase linearly with temperature up to 45°; the rate then decreased owing to protein (enzyme) degradation. Therefore, accurate temperature control was necessary. A temperature of 25° was used in our procedure as the incubation temperature, although the International Union of Biochemistry (8) now recommends 30° for activity measurements.

Results and Discussion

Representative calibration data from 50 coulometric determinations are shown in Table 1. Standards containing 23.2, 90.0, and 225.0 mg. glucose per 100 ml. gave a mean recovery of 62.6 ± 0.53% over a 1-week period. The recovery was reproducible from day to day and sample to sample. The calibration was linear over a concentration range of 25–450 mg./100 ml., with zero intercept. Sample size (25–100 μl.) did not affect the reproducibility. Analyses of different human serum samples and serum controls (Versatol-A) indicated that the precision of the method was not affected by the sample matrix.

Normal and abnormal serum glucose values usually fall within a range of 25–400 mg./100 ml. The coulometric method was thus capable of covering the entire anticipated range of serum glucose values. Routinely, the thiosulfate added was sufficient to reduce the iodine formed from the oxidation of 25–300 mg. glucose per 100 ml. (25 μl. serum). Concentrations below or above these limits were determined by appropriate increase in the generation current or dilution, respectively.

Having established its reliability for the determination of glucose in serum, the coulometric method was compared to other routine or standard methods now in use. A direct comparison of the precision, specificity, sensitivity, and ease of operation was sought. Three clinical procedures, the AutoAnalyzer, the Folin-Wu, and the Glucostat (Worthington Biochemical Corp., Freehold, N. J.) were selected. The AutoAnalyzer automatically dialyzes the protein from 0.5 to 1.0 ml. of serum and colorimetrically (ferricyanide reagent) determines the glucose. The Folin-Wu procedure (14) utilizes oxidation of glucose by
a copper reagent and subsequent colorimetric (phosphomolybdate) measurement. Enzymatic oxidation (glucose oxidase) of glucose is coupled with colorimetric determination of hydrogen peroxide (dye, peroxidase enzyme) in the Glucostat procedure. Comparison of the coulometric test was thus effected with automated, enzymatic, and reduction methods.

Table 2 gives results of samples analyzed by the various methods. Standards and serums were assayed to compare the colorimetric and AutoAnalyzer method. Three standards (parentheses indicate the source of the standard) indicated an uncertainty of 2% between these 2 methods. Representative data from 6 serum samples yielded AutoAnalyzer results averaging about 5 mg./100 ml. (4.5% relative) higher than the coulometric method. Sunderman and Sunderman (15) have reported that the AutoAnalyzer procedure averages 7 mg./100 ml. higher than glucose oxidase methods. One difficulty in assessing these results is the possible occurrence of glycolysis (e.g., by bacteria) during the time period (12 hr.) between AutoAnalyzer and coulometric assays. Sodium fluoride was added to inhibit glycolysis, and the samples were refrigerated immediately after the AutoAnalyzer runs. Coulometric data were taken immediately after warming the samples to 25°C. Considering these precautions, it would appear that the AutoAnalyzer test has a small positive bias. This positive error is probably due to the use of a nonspecific glucose reagent, ferricyanide.

The precision of the coulometric determination, even using 25 μl. compared to 1 ml., is 2–3 times greater than that reported for the AutoAnalyzer (7). The coulometric method could be easily automated to be competitive with the AutoAnalyzer system.

Table 2 also gives representative data from 9 serum samples analyzed by the coulometric, Folin-Wu, and Glucostat procedures in the authors’ laboratory. The samples were stored at 2°C prior to analysis. The 3 assay methods were successively performed on individual samples to eliminate possible differences due to standing. Lyophilized commercial control materials were reconstituted individually and assayed immediately for glucose. Coulometric data on Moni-Trol controls were compared to manufacturers’ assays via the specific procedures (Table 2). Sodium fluoride was not added to serum since Henry (7) has reported that fluoride may affect colorimetric-enzymatic methods that utilize peroxidase enzyme (Glucostat assay).

The reproducibility of the coulometric measurement was a factor of 2–3 times better than the other assays. This result is especially significant in the normal, fasting glucose range, 85 ± 20 mg./100 ml. (14). The precision obtained for the Glucostat and Folin-Wu tests was com-
Table 2. Determination of Serum Glucose

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose found (mg./100 ml.)</th>
<th>% Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coul.*</td>
<td>A.A.</td>
</tr>
<tr>
<td>Standard</td>
<td>(90.0)</td>
<td>88‡</td>
</tr>
<tr>
<td></td>
<td>(99.4)</td>
<td>(100)‡</td>
</tr>
<tr>
<td></td>
<td>(147.8)</td>
<td>(150)‡</td>
</tr>
<tr>
<td>Serum§</td>
<td>80.5</td>
<td>82‡</td>
</tr>
<tr>
<td></td>
<td>85.6</td>
<td>90‡</td>
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<td></td>
<td>99.5</td>
<td>103‡</td>
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<td>94.8</td>
<td>101‡</td>
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<td>109.7</td>
<td>117‡</td>
</tr>
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<td></td>
<td>173.5</td>
<td>180‡</td>
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<tr>
<td>Serum‖</td>
<td>88.5</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>100.4</td>
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<td></td>
<td>73.7</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>85.5</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>91.2</td>
<td>—</td>
</tr>
<tr>
<td>Moni-Trol I</td>
<td>103.5</td>
<td>105‡</td>
</tr>
<tr>
<td>Versatol-A</td>
<td>197.0</td>
<td>—</td>
</tr>
<tr>
<td>Moni-Trol II</td>
<td>243.0</td>
<td>277‡</td>
</tr>
<tr>
<td>Versatol-A</td>
<td>302.7</td>
<td>—</td>
</tr>
</tbody>
</table>

* 25-μl. sample; n = 3-5 (n is number of analyses per sample).
† 50-μl. sample, assays run in authors’ laboratory; n = 3.
‡ 1-ml. sample run at Walter Reed Army Institute of Research, Washington, D.C.; n = 1.
§ 0.5% (w/v) NaF added and samples refrigerated after A.A. runs; Coul. runs within 16 hr. after blood was sampled.
‖ No NaF added; samples stored at 2° and run in groups of 3.
¶ Manufacturer’s assay or added glucose.
** Manufacturer’s assay by Nelson-Somogyi method.

Comparable to reported data (7). The Folin-Wu (copper reduction) method averaged 10 mg./100 ml. higher than the coulometric measurement. The Glucostat determination averaged 4 mg./100 ml. less than the coulometric, with a difference range of −14 to +10 mg./100 ml. The range difference between the latter 2 methods is partially due to the colorimetric step of the Glucostat procedure. It was difficult to reproduce the absorbance reading of individual standards or samples to better than ±5%, relative. The literature reveals many modifications aimed at stabilization of the color in the Glucostat test. However, none of these appear to be very precise. In addition, accurate measurement and transfer of a PFF aliquot adds to the error. The Folin-Wu method, in addition to the aliquot error, requires accurately timed heating periods and cooling to room temperature before colorimetric measurement. Ease of operation was thus an advantage of the coulometric assay. Deproteinized samples were prepared, incubated, and titrated in the same cell and run at a convenient temperature (25°). No aliquots were required. Once the enzymatic reaction was stopped (adjustment to
pH 8.0), the samples could be titrated essentially at convenience. Coulometric data on analytically prepared serum controls confirmed literature data (7, 15) that enzymatic methods approach "true" glucose values. The data in Table 2 indicate that nonspecific reduction methods, in general, overestimate the glucose content.

Substances present in, or added to, serum that have been reported to interfere with glucose oxidase methods are as follows:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>&gt;5 mg./100 ml. interferes</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Not present in serum</td>
</tr>
<tr>
<td>Fluoride</td>
<td>2 gm./100 ml. did not interfere</td>
</tr>
<tr>
<td>Oxalate, thymol</td>
<td>0.5 gm./100 ml. (w/v) did not interfere</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Precipitated as zinc urate; 10 mg./100 ml. did not interfere</td>
</tr>
</tbody>
</table>

Ascorbic acid, normally present at 0.2–2.0 mg./100 ml., interfered with the coulometric method above 5 mg./100 ml. Addition of 1, 2, and 5 mg. ascorbic acid per 100 ml. to serum produced no interference. Preservatives added to inhibit glycolysis, e.g., fluoride, oxalate, and thymol, caused no interference. This was an important finding, since fluoride inhibition of glucose oxidase methods using peroxidase enzyme has been reported (7). Uric acid is precipitated as zinc urate; no interference was observed at 10 mg. of added uric acid per 100 ml. Additional evidence of the lack of interference of normal and abnormal serum constituents on the coulometric method was provided by the control serums. The Versatol-A and Moni-Trol samples reported in Tables 1 and 2 contained serum constituents at normal and abnormal levels. The close approximation of the coulometric determination of these commercial control materials provides evidence of the high specificity for glucose by glucose oxidase.

References