Development and Evaluation of a Glucose Analyzer for a Glucose-Controlled Insulin Infusion System (Biostator®)

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The Glucose-Controlled Insulin Infusion System (Biostator) is a modular, computerized, feedback control system for dynamic control of blood glucose concentrations in diabetics. This on-line glucose analyzer for use with whole blood utilizes a novel enzyme (glucose oxidase)-membrane configuration and an electrochemical cell to measure the \( \text{H}_2\text{O}_2 \) generated. The analyzer exhibits both short- and long-range stability, and instrument response and analyte concentration are linearly related over the full range of clinical interest. The response is fast, accurate, and precise, and permits determination of blood glucose within 2 min from the moment the blood leaves the patient. Correlation studies were completed to show the agreement between the Biostator Glucose Analyzer and the FDA's recommended hexokinase/glucose-6-phosphate dehydrogenase procedure on whole blood (e.g., average percent recovered for 11 concentrations between 250 and 9000 mg/liter was: hexokinase, 95.6%; Biostator Analyzer, 95.9%; bias and SD\( \text{D}_2 \), respectively, at low, normal, and high glucose values were: 12 and 41 mg/liter at the 500 mg/liter level; 4 and 52 mg/liter at the 1000 mg/liter level, and 4 and 128 mg/liter at the 4000 mg/liter level). No appreciable interference is observed with above-normal concentrations of bilirubin, uric acid, creatinine, sodium salicylate, or dextran. Platelet adhesion, which tends to decrease the useful life of the membrane, has been significantly decreased.

Additional Keyphrases: analytical systems • glucose in whole blood • monitoring diabetics • beta cell simulation

The Biostator® system is a computerized, modular system designed to simulate the function of the normal beta cell in the regulation of blood glucose through the use of glucose-controlled insulin infusion. This instrument (Figures 1 and 2) consists of the following modules: an analyzer pump to control the continual withdrawal and mixing of the blood; a glucose analyzer for the continual on-line analysis for blood glucose; a computer, programed with a set of algorithms that, depending on the dynamic and (or) static blood-glucose concentrations, calculates the amount of insulin and (or) dextrose to be infused; a computer-controlled infusion pump, to deliver the insulin and (or) dextrose to the patient; and a printer/plotter, which provides a minute-by-minute record of (e.g.) the glucose concentration and the amount of insulin infused. The heart of the Biostator system and one of the keys to successful feedback control of diabetics is obviously the glucose analyzer. The method chosen for measurement of blood glucose concentrations should be highly specific for glucose, have a fast response time, exhibit both short- and long-term stability, be linear over the range of interest, and be simple and inexpensive to operate. In early Biostator systems (1, 2) a modified continuous-flow system (Technicon AutoAnalyzer) was used, whereby the glucose was determined by either the glucose oxidase/peroxidase (EC 1.1.3.4/1.11.1.7) method or a hexokinase/glucose-6-phosphate dehydrogenase (EC 2.7.1.1/1.11.1.49) method. With this system there was a 5- to 6-min delay between withdrawing the blood and recording of glucose analysis by the computer. It also required about 70 ml of blood for a 24-h monitoring cycle, was relatively expensive in terms of reagent consumption, and occupied a lot of space at the patient's bedside.

In an attempt to overcome some of these objections, an effort was made to develop a new glucose analyzer module (2). The new analyzer was to be based upon an electrochemical sensor and a membrane with immobilized glucose oxidase. Specifically, a modified Clark-type electrode (3) is used whereby hydrogen peroxide, generated as the blood glucose reacts with the glucose oxidase is measured polarographically. The enzyme is immobilized in a novel enzyme-membrane configuration (4) covering the electrode. The membranes of this enzyme-membrane sandwich (Figure 3A) serve a dual purpose: they offer a means for immobilizing the enzyme and also serve as filters to block potentially interfering substances from reaching the electrode. The primary (outer) membrane acts as a prefilter, in that glucose can readily diffuse into the enzyme layer but macromolecules and cells are excluded. The secondary (inner) membrane is the fine filter and allows only the hydrogen peroxide generated by the enzyme reaction and salts of low relative molecular mass to reach the electrode. Potentially interfering substances with a

1 Life Science Instruments, Miles Laboratories, Inc., Elkhart, Ind. 46515.
2 Ames Division, Miles Laboratories, Inc., Elkhart, Ind. 46515.
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3 Clemens, A. H., and Chang, P. H., Development of an on-line rapid blood glucose analyzer (RBGA) in a glucose controlled insulin infusion system (GCIIS). Diabetes 25 (Suppl. 1), 358 (1976). Abstract.
relative molecular mass greater than 250 are efficiently blocked. The flow-through cell (Figure 3 B and C) was then designed to provide a small dead volume, a short washout time, optimum exposure of the solution to the electrode, elimination of static noise pickup, and a simple, trouble-free membrane-loading procedure.

The present Biostator Glucose Analyzer meets all of the requirements outlined. It has shortened to 90 s the lag time between blood withdrawal and determination of blood glucose concentration for use by the computer; decreased the blood-consumption rate to 50 ml/24 h; decreased the cost of operation through the use of an immobilized enzyme system; and, owing to its compact size, has enabled the entire Glucose-Controlled Insulin Infusion System to fit into a cabinet the size of a portable television set.

Here, we describe the results of an evaluation of this glucose analyzer, including a two-part correlation study showing the agreement between results from it and the proposed National Glucose Reference Method (5) for more than 170 samples of whole blood.

**Materials and Methods**

**Procedure for Biostator Glucose Analyzer:** Analysis for blood glucose on the Biostator instrument is accomplished by an electrochemical sensor, enzyme-membrane system (Figure 3). The diluted blood is drawn into the probe and the following reactions take place:

1. **D-Glucose + O₂ → D-gluconic acid + H₂O₂**
   
   \[
   \text{glucose oxidase} \quad \text{Pt anode} \quad 0.7V
   \]

   \[
   \text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2\text{e}^-
   \]

   The current generated then serves as a measure of the glucose content. The flow scheme for the analyzer (Figure 4) permits complete calibration without disconnecting the double-lumen catheter from the patient. An initial two-point calibration is performed by using a baseline solution (BASE) and a glucose standard (STD) equivalent to a diluted 2.20 g/liter glucose solution (Life Science Instruments). The calibration procedure is then
completed by performing a modified standard-addition procedure (6–9) on the whole-blood sample. One part of blood is diluted with 10 parts of each of six glucose standards (Analyzer Glucose Addition Calibration Kit; Life Science Instruments). The standards are such that, when diluted with the blood, 1,00 mg/liter increments in glucose concentration are observed between 0 and 5.00 mg/liter (Figure 5). The observed concentration readings are plotted vs. the known glucose content of the diluted standard (Figure 6). A linear-regression analysis is performed and the glucose concentration (G) of the blood sample is calculated (8) by equation 3:

\[ G = \text{intercept}/\text{slope} \]  

This in vitro calibration step, which determines the blood glucose concentration as well, is done through the sample valve (Figure 4). The results are then used to make adjustments to the “pump dilution ratio” and thereby match the on-line reading for blood glucose with the calculated in vitro value.

All standards and diluents used in conjunction with the Biostator system are buffered at pH 7.0 ± 0.2 and have an ionic strength of 0.15 mol/liter, thereby preventing any hemolysis. The formulation for the BASE solution and all of the glucose standards was derived to duplicate the blood diluents used. This then cancels any potentially adverse effect which any of the components of the buffers may have.

For part one of the correlation study the glucose addition procedure was run in triplicate on each sample. Air displacement micro-pipets (e.g., Eppendorf pipets) were used for handling the standards (1000 μl) and the blood (100 μl). The glucose-addition procedure was completed in duplicate during the second part of the correlation study and a 100-μl positive-displacement type of micro-pipet (e.g., Drummond) was used to handle the blood.

Procedure for Glucose Reference Method: The proposed National Glucose Reference Method (5) was used during both parts of the correlation studies. This method is based on the following scheme:

\[ \text{D-Glucose} + (\text{ATP} \cdot \text{Mg})^2^- \xrightarrow{\text{hexokinase}} \text{D-glucose-6-phosphate} \]
\[ + (\text{ADP} \cdot \text{Mg})^- + H^+ \]  

(4)

\[ \text{D-Glucose-6-phosphate} + \text{NAD}^+ \xrightarrow{\text{dehydrogenase}} 6\text{-phosphoglucono-delta-lactone} \]
\[ + \text{NADH} + H^+ \]  

(5)

For each mole of glucose present in a deproteinized sample, one mole of NAD\(^+\) is reduced to NADH and the resulting increase in absorbance at 340 nm is followed spectrophotometrically. The procedure was performed in duplicate on all samples as previously described (5), except that samples of heparinized whole blood were used in place of serum or plasma. In doing so, the deproteinization step was completed on the whole blood within 1 h of sample preparation. This also served to halt any glycolysis. Blood samples with a glucose content greater than 3.00 g/liter were diluted with distilled water according to the following scheme: 4.00 and 5.00 g/liter samples diluted two-fold, 6.00 and 7.00 g/liter samples diluted threefold, and 8.00 and 9.00 g/liter samples diluted fourfold. The hexokinase (from yeast, EC 2.7.1.1.), the NAD, and the ATP were purchased from Calbiochem, San Diego, Calif. 92112, and the glucose-6-phosphate dehydrogenase (from L. mesenteroides) from Worthington Biochemicals, Freehold, N.J. 07728. All other chemicals were reagent grade.
Absorbances were measured with a Model 300 Spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio 44074).

Sample preparation: Part one of the correlation study involved measuring the blood glucose of 61 different healthy men and women volunteers during 10 days. About 15 ml of blood was collected from each volunteer into heparinized Vacutainer Tubes (Becton-Dickinson, Rutherford, N.J. 07070). Three samples were assayed each day and the results were assumed to represent normal concentrations. Of the remaining three or four samples taken each day, one was allowed to undergo glycolysis for 3–4 h in an attempt to generate a low value, and various increments of a 100 g/liter glucose solution were added to the other samples, to generate high values. We were not concerned with analytical recovery at this time, so it was not necessary to know the initial glucose concentration of these samples.

Part two of the correlation study involved assaying 110 contrived samples (10 at each of the following glucose concentrations: 0.25, 0.50, 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, and 9.00 g/liter) by each method. Microburets (0.2 ml and 2.0 ml; Roger Gilmont Instruments, Inc., Great Neck, N.Y. 11021) were used to make additions of a 100 g/liter glucose (2 g of benzoic acid added per liter as preservative) to 10.0 ml of glycolyzed whole blood. About 150 ml of blood was collected from healthy volunteers, pooled, and allowed to undergo complete glycolysis for 16–24 h at 37 °C. Use of blood with essentially zero glucose as a starting point was necessary for controlling the very low concentrations.

Glycolysis, as indicated above, was halted by the deproteinization step in the reference method. For the Biostator procedure a preliminary study showed that once the blood samples were diluted with glucose standards and stored at 4 °C the amount of glycolysis was held to less than 2% for as long as 6 h.

For the interference study, we added increments of a concentrated solution of the potential interfering substance (10) to either samples of normal whole blood or a commercial serum standard (Validate; General Diagnostics, Morris Plains, N.J. 07950). The increments to final concentrations (per liter) were: bilirubin (Eastman Kodak Co., Rochester, N.Y. 14650) 100 mg; creatinine (ICN Pharmaceuticals, Cleveland, Ohio 44128) 100 mg; uric acid (ICN Pharmaceuticals) 100 mg; sodium salicylate (Fisher Scientific, Pittsburgh, Pa. 15219) 350 mg; and dextran, clinical grade (ICN Pharmaceuticals) 10 g. Samples with the increased concentrations of each substance were then compared to a blank blood or serum sample.

Results and Discussion

Part one of the correlation study was completed essentially as a patient-sample study in which the results of the glucose analyzer method were compared to those of the reference method. One of the most accepted formats for analyzing such data is a plot (Figure 7) of the test method results vs. the reference method (11–13). In addition, a least-squares analysis was performed, in which the calculated parameters served as an estimate of the various types of errors (13). In this case, the slope of 1.00 indicates that the system is free of proportional error; the $S_y$ (standard error of estimate in the y direction) implies a random error of 79 mg/liter, and the y-intercept suggests that there is a bias or constant error of $-49$ mg/liter. The correlation coefficient, 0.996, is nearly ideal. In reference to the bias or constant error, we believe that a significant portion of this may be attributed to the fact that air-displacement pipets were used to deliver the blood. A recent report (14) suggests that the use of these pipets on whole blood may result in inaccuracies in the delivery of the intended volume. Primarily for this reason we used the positive-displacement type of pipet (SMI or Drummond) in part two of the study. As will be seen, this change in pipets resulted in a significant decrease in the bias.

Each sample analysis was completed in triplicate on the Biostator, and the average value was then compared to results by the reference method. The average standard deviation for the triplicate assays for each sample was only 2.3%. Considering the pipets used, we believed that this variation was small enough to require only duplicate assays for each sample during the second part.

Part two of the correlation study involved assaying contrived whole-blood samples at discrete concentrations over a rather extended range (0.25 to 9.00 g/liter). This permitted an evaluation of the Biostator Glucose Analyzer at each glucose concentration (Table 1). The guidelines of the FDA's proposed Product Class Stan-

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4 To include changes as reported in April 13, 1976, FDA draft of the Proposed Product Class Standard for the Quantitative Measurement of Glucose in Serum or Plasma and those recommended by the FDA Chemistry Advisory Subcommittee on April 26 and 27, 1978.
Table 1. Results of Correlation Study, Part II

<table>
<thead>
<tr>
<th>Target glucose concn., mg/liter</th>
<th>Mean value</th>
<th>Bias a/SD d b</th>
<th>Recovered, % (target value)</th>
<th>Recovered, % of reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/liter</td>
<td>n</td>
<td>Ref./Biostator</td>
<td>mg/liter</td>
<td>Ref./Biostator</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------</td>
<td>---------------</td>
<td>-----------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>250</td>
<td>10</td>
<td>186/170</td>
<td>16/52</td>
<td>74/68</td>
</tr>
<tr>
<td>500</td>
<td>10</td>
<td>442/414</td>
<td>28/59</td>
<td>88/83</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>945/941</td>
<td>4/52</td>
<td>95/94</td>
</tr>
<tr>
<td>2000</td>
<td>9</td>
<td>1965/1942</td>
<td>19/52</td>
<td>98/97</td>
</tr>
<tr>
<td>3000</td>
<td>9</td>
<td>2934/2973</td>
<td>-39/104</td>
<td>92/102</td>
</tr>
<tr>
<td>4000</td>
<td>10</td>
<td>3997/3994</td>
<td>4/128</td>
<td>100/100</td>
</tr>
<tr>
<td>5000</td>
<td>10</td>
<td>4938/5084</td>
<td>-146/211</td>
<td>100/100</td>
</tr>
<tr>
<td>6000</td>
<td>10</td>
<td>6012/6092</td>
<td>-79/216</td>
<td>100/100</td>
</tr>
<tr>
<td>7000</td>
<td>10</td>
<td>6973/7135</td>
<td>-162/271</td>
<td>102/102</td>
</tr>
<tr>
<td>8000</td>
<td>10</td>
<td>7937/8201</td>
<td>-269/616</td>
<td>103/103</td>
</tr>
<tr>
<td>9000</td>
<td>10</td>
<td>8964/9385</td>
<td>-421/835</td>
<td>104/104</td>
</tr>
</tbody>
</table>

a Bias = difference between means (Reference - Biostator).
b SD d = standard deviation of differences = \([a(x - y - \text{bias})/n - 1]^{1/2}\).

dard for Glucose (10) were used to assist in this evaluation. In accordance with these guidelines, the limits of acceptability are expressed as agreement between the reference method and the test method within 5% or 50 mg/liter, whichever is greater, for both precision (standard deviation) and accuracy (bias). The data in Table 1 reveal that the accuracy (bias) requirement is well within the suggested limits over this entire range of concentrations. The precision (measured as SD d, the standard deviation of the differences) is at the borderline value between 250 and 1000 mg/liter, considerably below the 5% requirement in the 2 to 7 g/liter range and exceeds the limit at the 8 and 9 g/liter concentrations. The SD d value of 59 mg/liter of the 500 mg/liter concentration is elevated because of a sample with an individual bias of 160 mg/liter (reference value 500 mg/liter, Biostator value 340 mg/liter). If this case is omitted, the mean bias becomes 12 mg/liter and the SD d value 41 mg/liter. At the other end of the spectrum, the large SD d values at 8 and 9 g/liter are due primarily to loss of linearity during the glucose-addition calibration procedure. The linearity of the glucose sensor tends to tail off at high concentrations, thereby reducing the slope, which in turn results in larger G values (equation 3). This large amount of random error at 8 and 9 g/liter is also observed in Figure 8.

A least-squares analysis performed on the results in the 0.50 to 4.00 g/liter range of Figure 8 shows an improvement over the same range covered in Figure 7 (part one), i.e., slope = 1.01, y intercept = -19.5 mg/liter, and r = 0.998. Both Figures 7 and 8 show that the Biostator Glucose Analyzer exhibits very good linearity over the range of normal clinical interest (0.50 to 4.00 g/liter).

The format used for part two also permits an assessment of the percentage recovered (Table 1). Note that at 250 and 500 mg/liter neither method accounted for more than 90% of the target value (probably indicative of problems in contriving the samples). To compensate for potential errors in sample preparation, the last column of Table 1 shows the value by the Biostator procedure as a percentage of the value found by the reference method, the average being 100.1%.

The two remaining suggested performance requirements (10) are the incidences of excessive individual

Fig. 8. Plot of results of part two of the correlation study (Biostator vs. Proposed National Reference Method)

Table 2. Results of Interference Study

<table>
<thead>
<tr>
<th>Interfering substances</th>
<th>Added concn. interval</th>
<th>Blood glucose Blank</th>
<th>Sample mg/liter</th>
<th>Serum glucose Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>100</td>
<td>1440</td>
<td>1430</td>
<td>960</td>
<td>940</td>
</tr>
<tr>
<td>Creatinine</td>
<td>100</td>
<td>1450</td>
<td>1450</td>
<td>910</td>
<td>910</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>350</td>
<td>1430</td>
<td>1430</td>
<td>910</td>
<td>920</td>
</tr>
<tr>
<td>Uric acid</td>
<td>100</td>
<td>1480</td>
<td>1470</td>
<td>980</td>
<td>970</td>
</tr>
<tr>
<td>Dextran</td>
<td>10 000</td>
<td>1420</td>
<td>1430</td>
<td>950</td>
<td>940</td>
</tr>
</tbody>
</table>
bias and interference from other substances. Excessive individual bias is defined as a difference between the reference-method value and the test-method value greater than 15% or 150 mg/liter, whichever is larger. The allowable number of cases of excessive individual bias is 5%. Of the 170 samples run in parts one and two, there were only four cases of excessive bias (reference method value/Biostator value, in g/liter: 0.50/0.34, 8.92/10.58, 7.95/9.87, and 8.30/10.85). Note that for three of the four cases the biases were for the 8 and 9 g/liter concentrations.

We determined the effect of five potentially interfering substances: bilirubin, creatinine, uric acid, dextran, and sodium salicylate. In accordance with the guidelines (10) no substance should result in a 50 mg/liter difference. The results (Table 2) show that none of the substances tested has a substantial effect. Interference from other substances (e.g., ascorbic acid, heparin, etc.) is eliminated by the membrane system (4); variations in blood pH or CO₂ content have no effect, owing to the buffer capacity of the diluents. As indicated, the electrode is responsive to H₂O₂ and insensitive to O₂ (3).

The Biostator system is intended for the continuous on-line analysis for glucose in whole blood, and as a result the sensor is exposed to blood for about 85% of its operating time. Initially this led to a brief useful lifetime for the membrane, owing to adhesion of platelets and erythrocytes (Figure 9 A and B). However, through the use of specially formulated diluents the problem of platelet adhesion has been significantly diminished (Figure 9C) and the membranes now have an average useful lifetime of 25–50 h.

In conclusion, the above results clearly indicate that the Biostator Glucose Analyzer can precisely and accurately measure glucose in whole blood, and is well suited for use as a continual on-line glucose analyzer to aid in the control of diabetics.

References


