METHODS

Measurement of Glucose Concentrations in Human Blood Sera with a Bacterial Biosensor

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A laboratory model of a bacterial sensor based on Clarke's electrode is used to measure the glucose concentration in human blood serum. The results of the measurements are compatible with the data of standard blood glucose measurement using the color reaction with orthotoluidine, which is used in medical biochemical laboratories, and with the results of measurements by means of the commercial glucose analyzer Eksan-G. The coefficients of correlation with the data of the glucose analyzer and of the routine method of glucose measurements were 0.97 in both cases. The accuracy of glucose measurement by the bacterial sensor is within 2%.

Key Words: *bacterial biosensor; amperometric detection; glucose; human blood*

Studies aimed at designing biosensors for medical diagnosis have recently made great strides. For example, in the measurement of the glucose concentration in the blood (an important clinical test for diabetes) close attention is paid to blood glucose monitoring *in vivo* [3]. The efficacy of new types of transformers [7] and modifications of methods for enzyme immobilization [6] are being investigated. Highly selective mediatory electrodes have been described [1].

The above models, as well as the majority of others, are based on the use of glucose oxidase in the receptor element, although this is not the only variant of creating biosensor devices of this type. A promising trend of research is the development of bacterial biosensors whose sensitivity is based on the enzymatic activity of microorganisms [4,9]. Potentiometric bacterial biosensors based on pH-controllers for glucose measurement have been described [4,8]. A serious drawback of potentiometry is the fact that the method's sensitivity depends on the buffer properties of the measured medium, which is why this method is not yet widely used for analysis of blood samples with a high buffer capacity. Amperometric converters are free of this flaw.

Previously we demonstrated the possibility of using immobilized *Gluconobacter oxydans* cells as a receptor of a bacterial potentiometric sensor for glucose measurements [4].

This study explored the possibility of using a model of bacterial sensor (BS) based on amperometric measurement of glucose in serum samples. Our goal was to study the sensitivity of BS in measurements of glucose in reference solutions and

TABLE 1. Coefficients of Correlation of Data Obtained by Different Methods

	BS	OM	Eksan-G
BS	1.00	0.97	0.97
Eksan – G	0.97	0.96	1.00

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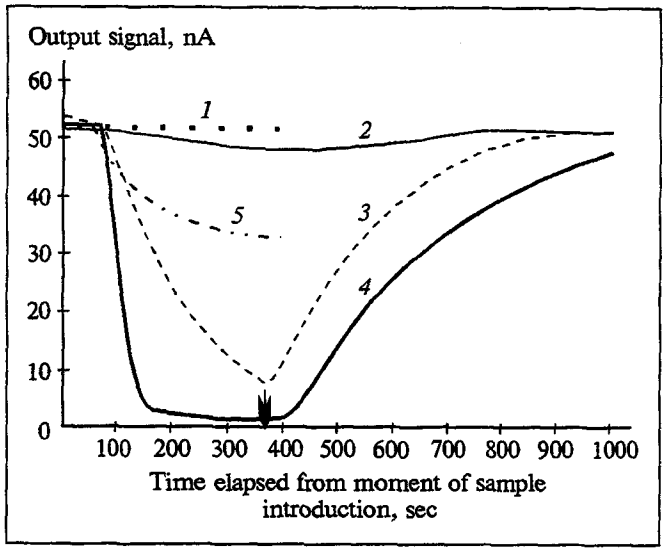

Fig. 1. Typical BS responses to the introduction of reference glucose solutions and serum samples to the cuvette. Glucose concentration: *1*) 0.03, *2*) 0.17, *3*) 3, *4*) 30 mM; *5*) serum sample, 2.08 mM. The arrow shows the moment when washing started.

serum samples and to compare the proposed detection method with known analogs.

MATERIALS AND METHODS

Glucose was measured in the blood using the color reaction with orthotoluidine (orthotoluidine method - OM) as described previously [2]. This method is routinely used in medical biochemical laboratories.

Glucose was measured in the blood using a commercial biosensor analyzer Eksan-G based on glucose oxidase and a peroxide amperometric electrode according to the manufacturer's instructions.

BS was represented by Clarke's oxygen electrode (Ingold) with *Gluconobacter oxydans* cells immobilized on the membrane surface.

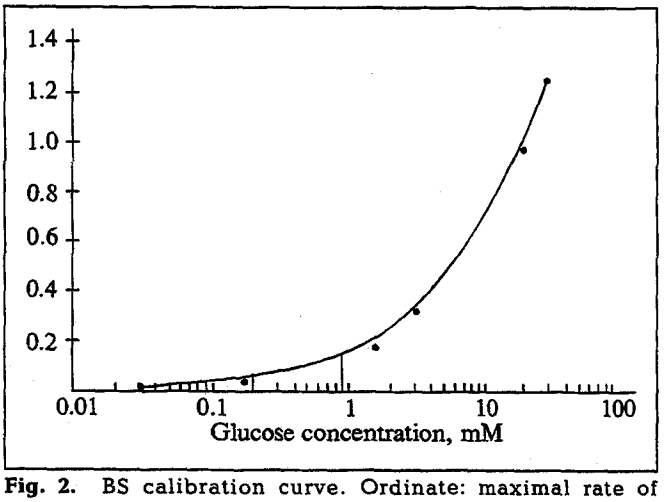

Fig. 2. BS calibration curve. Ordinate: maximal rate of changes in dI/dt output signal, nA/sec. Vertical lines: working range of glucose concentrations.

The biomass was prepared as described previously [4]. Immobilization of cells by adsorption was achieved with a suspension in sterile tap water containing 100 mg wet cells per ml. Suspension (10 μl) was sprayed onto the electrode surface with a Hamilton microsyringe, dried, and fixed with a capron net. The measurements were carried out in an open 5-ml cuvette with 20 mM sodium phosphate buffer (pH 6.8) at 18°C and constant stirring.

For plotting of the calibration curve in the concentration range of 0.01 to 10 mM, portions of 1, 0.1, or 0.01 M glucose solution were placed in the cuvette so as to preset the substrate concentration in the medium. The parameters measured were the maximal rate of changes in the output signal of the electrode dI/dt (nA/sec), which is proportional to the rate of changes in the oxygen concentration in the layer of immobilized cells, and the amplitude (nA) of the output signal defined as the difference between the initial current value when there is no glucose in the cuvette and the current value recorded at a certain moment after the addition of glucose. After the signal had been recorded, the electrode and cuvette were washed with phosphate buffer. For measuring glucose concentrations in the serum, samples of 500 μl were added to the cuvette and the glucose concentrations were found on the calibration curve. All measurements were carried out in the 80-100% range of oxygen saturation of the environment.

The data were mathematically processed as recommended elsewhere [5]. Serum samples were obtained from the biochemical laboratory of Pushchino-on-Oka Medical Center.

RESULTS

A typical response of BS to the addition of glucose in different concentrations in the cuvette is presented in Fig. 1. As the glucose concentrations increased, the initial rate of changes in the output signal and its amplitude also increased. The time of BS recovery, defined as the time needed to attain the initial level of the signal preceding the addition of a sample, depended on the glucose concentration in the sample and varied from 2 min for low (0.01 mM) to 15 min for high (10 mM) glucose concentrations (Fig. 1).

The accuracy of the measurements (reproducibility of the results) was assessed by measuring BS signals upon the addition of glucose in a concentration of 1 mM seven times. Relative errors in measurements determined for the rate of signal change dI/dt and its amplitude were 1.39 and

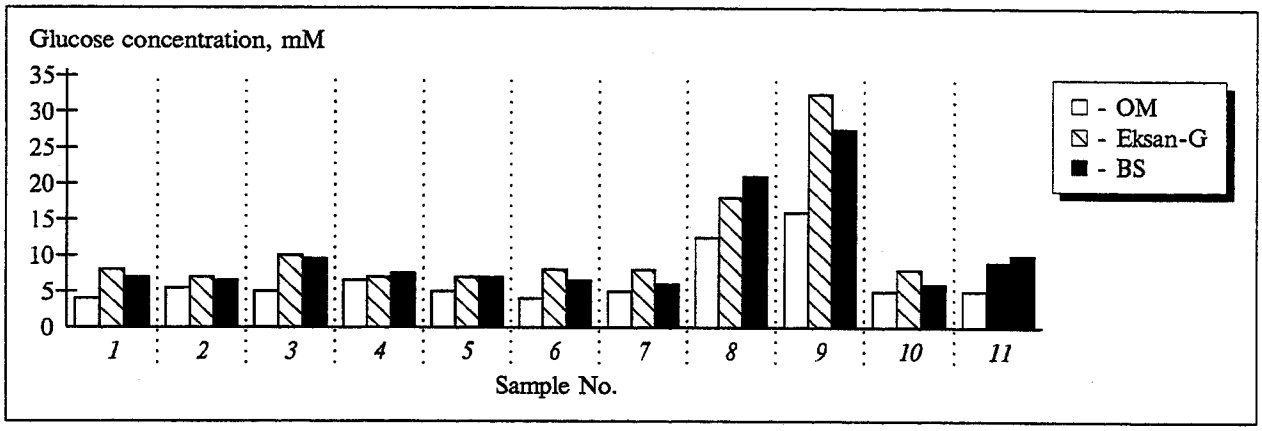

Fig. 3. Results of glucose analysis in serum samples obtained by different methods.

1.62%, respectively, and the standard deviations (σ_{n-1}) were 0.0062 and 1.02, respectively.

A calibration curve of BS plotted on the basis of the relationship between the maximal rate of signal change and the glucose concentration is presented in Fig. 2. The minimal glucose concentration recorded was 0.02 mM. The results of glucose measurement in serum samples obtained using BS, the Eksan-G analyzer, and OM are presented in Fig. 3 (measurements of the concentrations by OM were carried out in the biochemical laboratory of the Medical Center). The concentrations obtained by biosensor measurement were in general higher than the values obtained by OM. A quantitative assessment of the correlation between the data of BS and OM and of BS and the Eksan-G analyzer is presented in Table 1.

Two series of experiments were carried out for a more detailed assessment of the possibilities of the glucose analyzer we designed and for comparing the data of the orthotoluidine and biosensor methods. In the first series a preset glucose concentration was measured by three methods in a model medium, 50 mM phosphate buffer, pH 6.8 (Table 2). The data obtained by biosensor analyzers virtually coincided; OM yielded somewhat lower values. The second series of experiments was aimed at verifying the accuracy of BS measurements of glucose concentrations which were increased in the samples by a certain magnitude. In this series of experiments certain quantities of glucose were added to blood samples with known concentrations of glucose measured by BS and Eksan-G, and the new concentrations were estimated (Table 3). The

TABLE 2. Assessment of Glucose Content in Phosphate Buffer (20 mM, pH 6.8) by Different Methods ($M \pm m$, 5 measurements)

Glucose concentration, mM	Concentration estimates, mM		
	OM	Eksan-G	BS
5	4.8±0.57	5.19±0.46	5.23±0.09
10	9.2±1.10	10.23±0.38	10.31±0.19
15	12.9±1.54	14.88±0.92	14.88±0.28
Relative error	11.96%	5.27%	1.92%

TABLE 3. Assessment of the Accuracy of Measurements by Biosensors for an Increase of Glucose Concentrations by a Certain Value (10 mM)

Type of biosensor	Concentration estimate, mM		Change in concentration, mM	Mean change of concentration	Standard deviation (σ_{n-1})
	initial	final			
Eksan–G	2.26	12.0	9.74		
	6.83	16.66	9.83	9.80	0.06
	8.48	18.32	9.84		
BS	2.53	11.7	9.17		
	6.32	15.85	9.53	9.37	0.18
	7.76	17.17	9.41		

error in the determination of the concentration of glucose added to the sample was 1.92% for BS, which is no higher than for Eksan-G (2.36%).

The results suggest that the proposed BS model is highly sensitive, and so can be used for measuring glucose concentrations ten times lower than the normal concentration in the blood, hence allowing glucose measurements to be performed in 10-fold diluted blood. The relative error of measurements is of the order of 2%. Serum components evidently exert no toxic effects on the bioreceptor element and do not disrupt its normal functioning; besides glucose, the blood does not contain any other components which might be taken for substrates by BS and thus lead to erroneous results. Analysis of a sample takes 2-3 min, followed by a 4-6 min recovery of BS for analysis of the next sample.

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REFERENCES

1. S. V. Bobrin, P. Atanasov, I. Iliev, *et al.*, *Elektrokhimiya*, **29**, № 8, 1013-1016 (1993).
2. V. G. Kolb and V. S. Kalinnikov, *Handbook of Clinical Chemistry* [in Russian], Minsk (1982).
3. D. Mascone, H. Yamanaka, and M. Maschini, *Elektrokhimiya*, **29**, № 12, 1528-1553 (1993).
4. A. N. Reshetilov, M. V. Donova, and K. A. Koshcheenko, *Priklad. Biokhim.*, **28**, 518-524 (1992).
5. E. Lloyd *et al.* (Eds.), *Handbook of Applicable Mathematics*, Wiley (1980).
6. M. R. Tarasevich, V. A. Bogdanovskaya, and G. V. Zhutaeva, *Elektrokhimiya*, **29**, № 12, 1554-1559 (1993).
7. A. A. Shul'ga, S. V. Dzyadevich, A. P. Soldatkin, *et al.*, *Ibid.*, **29**, № 8, 998-1002 (1993).
8. J. Racek, *Appl. Microbiol.*, **34**, № 4, 473-477 (1991).
9. K. Riedel, R. Renneberg, U. Wollenberg, *et al.*, *J. Chem. Technol. Biotechnol.*, **44**, 85-106 (1989).