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A CONTINUOUS MONITORING SYSTEM FOR BLOOD GLUCOSE MEASUREMENTS IN CONSCIOUS ANIMALS WITHOUT SURGERY

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ABSTRACT

The hypoglycemic activity of insulin delivered via the various routes is commonly determined by measuring glucose levels in the whole blood samples taken intermittently at frequent intervals. This procedure is usually performed in the anaesthetized animals, however, the glucose levels determined could be higher than those in the conscious animals. Furthermore, the animals are usually excited by direct contact with the investigator during the frequent blood sampling in conscious state, which could cause the glucose level to be higher than at undisturbed state. To overcome these problems, a continuous blood glucose monitoring system was developed by connecting the sensor chamber of a glucose analyzer (YSI Industrial Analyzer model 27), which utilizes glucose oxidase for glucose measurement, to a peristaltic pump with a specially designed mixing chamber and a data acquisition station. By using this system, the hyper-/hypo-glycemic activity of glucagon/insulin formulations in the conscious animals, after various routes of administration, can be determined without any interruption and/or disturbance.

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INTRODUCTION

The accurate determination of the hypoglycemic potency of an insulin product requires measurement of glucose levels in either blood or plasma samples taken by intermittent sampling at frequent intervals [1-3]. This procedure is usually performed in the anesthetized animals, which may yield higher glucose levels than in the conscious animals. The animals, at conscious state, are often disturbed by frequent blood withdrawal, which could cause the glucose levels to fluctuate and be different from that with no disturbance. Furthermore, even under highly standardized conditions, blood glucose levels can change rapidly and extensively over successive nyctohemeral cycles, especially in the most unstable diabetics [4,5]. Therefore, development of a continuous glucose monitoring system for monitoring these rapid changes in blood glucose concentration with minimal disturbance to the conscious animals is critically important.

The idea of developing a continuous glucose monitoring system can be traced back as early as 1960 [6,7]. Most of the earlier systems used an AutoAnalyzer, for continuous colorimetric analysis [6-10]. Unfortunately, the ferricyanide used in the AutoAnalyzer also measures the nonglucose-reducing substances in the blood [11, 12]. A polarographic device was developed for measuring polarographically-inactive glucose by converting it, using a glucose oxidase-coating electrode, into polarographically-active product [13-15]. It was improved later with the invention of a glucose oxidase-containing laminated membrane [16,17] to prevent the passage of interfering substances, which has formed the basis for the development of Yellow Springs model Glucose Analyzer. This glucose oxidase laminate was also applied to fabricate a glucose-sensor probe used in a continuous glucose monitoring system [18], which has improved the specificity of glucose measurement over the AutoAnalyzer [11,18]. A major technical problem has been the prevention of blood coagulation withdrawn [6-10]. To solve the problem, most of the earlier systems utilized a double-lumen cannula: one for withdrawing blood and another for adding heparin to the blood withdrawn [6-10]. A simple heparinization technique was developed to make a surface nonthrombogenic polymers [19]. This technique was successfully applied to fabrication of a nonthrombogenic intravenous catheter for continuous

withdrawal of blood [20], which permits the blood glucose levels to be monitored continuously without administration of heparin [11,18].

Further studies in the field has resulted in the development of a computer-controlled continuous glucose monitoring system [21,22]. The continuous glucose monitoring systems have been reported in the literature [6-10,13-15,18,20-22], which were designed either for human or for animal application. Although some of them can be used in a conscious human, however, they require surgery and/or anesthesia for use in animals. In this laboratory, we have further modified the system for continuous monitoring of blood glucose in the conscious animal without surgical manipulation [6,22]. Using this modified system, the blood glucose levels in the unanesthetized animals can be continuously measured without any interruption and/or disturbance. For optimization of this system for various animals, studies were performed to characterize various system parameters. Thereafter, the rabbit was chosen as the animal model. Due to the limitation of its blood volume, small sample withdrawing tubing and slow pumping speed are preferred. However, slowing the pumping speed, will delay response time and increase the occurrence of blood coagulation. To overcome these problems, large buffer withdrawing tubing is required. Based on the results obtained during the characterization of various system parameters, an optimal combination of parameters can be achieved. Thereafter, studies were performed to validate these optimized parameters selected for rabbit model. The results were compared, under the same condition, with that attained by conventional intermittent sampling technique in the same rabbits, at conscious state, following an intravenous administration of insulin.

MATERIALS AND METHODS

Materials

YSI Industrial Analyzer (Model 27), glucose oxidase membrane, glucose standard, and buffer solution (for the Industrial Analyzer) were purchased from Yellow Springs Instrument (Yellow Springs, Ohio). Peristaltic pump and tubings (for the pump) were obtained from Cole-Parmer Instrument Co. (Niles, Illinois). PE-10 (non-radiopaque polyethylene micro-tubing) was from Clay Adams (Division of Becton Dickinson, Parsippany, New Jersey). Sterile Water For Injection, Heparin

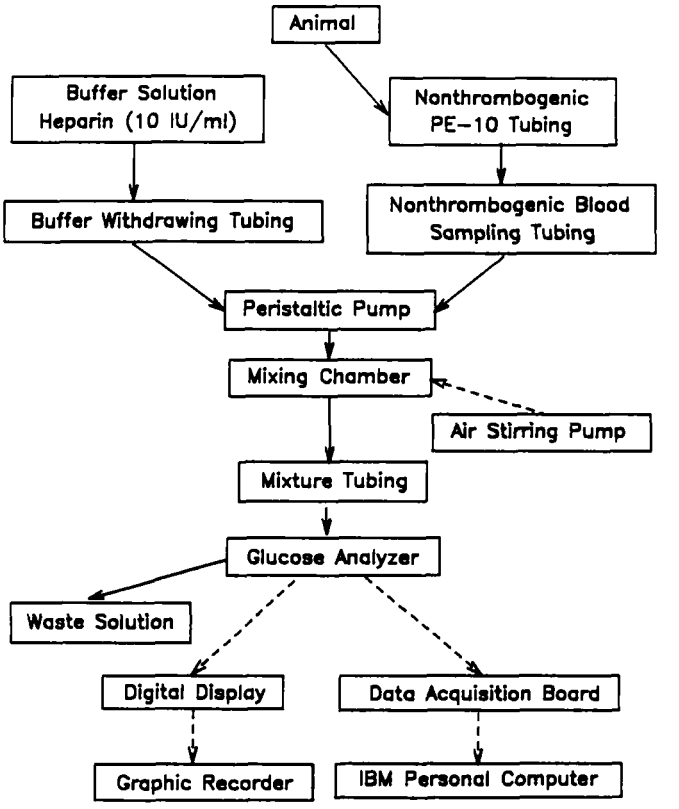

FIGURE 1
Schematic illustration of various components constituting the continuous glucose monitoring system and flow chart.

Lock Flush Solution (100 USP units/ml), and Butterfly Intermittent Infusion Set were supplied by Abbott Hospital, Inc. (North Chicago, Illinois). Tridodecylmethylammonium Chloride-Heparin complex (TDMAC-Heparin) was obtained from Polysciences, Inc. (Warrington, Pennsylvania). Humulin® R (regular recombinant human insulin solution) was purchased from Eli Lilly & Co. (Indianapolis, Indiana).

Instruments

The continuous blood glucose monitoring system developed for this investigation was assembled by connecting the sensor chamber in the glucose analyzer with a peristaltic pump, a specially-designed mixing chamber, and a data acquisition station.

The system is essentially composed of three stations in series: one for blood sampling and mixing with buffer solution, one for blood glucose measurement, and one for data acquisition. A flow chart illustrating the whole system is illustrated in Figure 1.

1) The blood sampling/buffer mixing station is composed of a peristaltic pump, a Plexiglas mixing chamber, a air stirring pump, and a network of interconnecting nonthrombogenic tubings for blood withdrawing and buffer withdrawing. The inside wall of these tubings that come into direct contact with blood samples was coated by TDMAC-heparin [19] to create a nonthrombogenic surface. The blood was withdrawn continuously from the rabbit's ear vein via the nonthrombogenic tubing at a fixed rate, by the peristaltic pump, and transported to the Plexiglas mixing chamber, where it was mixed with buffer solution [23] (containing 10 IU/ml of heparin) withdrawn simultaneously via the buffer withdrawing tubing from a buffer solution container. The specially-designed mixing chamber was fabricated from in two pieces of Plexiglas with a silicone diaphragm sandwiched in-between and connectors for blood and buffer withdrawing tubings. In the mixing chamber, blood sample is mixed thoroughly with buffer solution by the vibration of the silicone diaphragm produced by an oscillating air stream of the air stirring pump. Immediately after mixing, the mixture was transported into the sensory chamber of glucose-measuring station for measuring glucose concentration in the blood sample by glucose oxidase method.

2) The glucose-measuring station is utilized a YSI Industrial Analyzer, which containing a glucose-sensing probe, to carry out the glucose measurement [23]. The digital display of the glucose analyzer and a graphic recorder, which connecting to the output port of glucose analyzer, were used to display and record the glucose concentration. The glucose-sensing probe is a Clark electrode [15,23], whose tip is covered with a membrane impregnated with glucose oxidase and its cathode can be polarized by application of a constant voltage. Glucose was oxidized enzymatically by the oxidase as it diffused through the glucose oxidase-impregnated membrane, which generated hydrogen peroxide at a rate proportional to the concentration of glucose [23]. The hydrogen peroxide then oxidized the polarizing layer of hydrogen,

which permits current to pass through the probe [23]. The current flowing through the electrode is a linear function of the glucose concentration in the sample [23].

3) The data acquisition station is composed of a IBM computer, a data acquisition board (catalog number: L-08302-00, Cole-Parmer Instrument Company, Niles, Illinois), a terminal panel (catalog number: L-08303-20, Cole-Parmer Instrument Company, Niles, Illinois), and a software program named QuickLog PC (Strawberry Tree Incorporated, Sunnyvale, California). The data acquisition board interfaces with the IBM computer and the terminal panel which is connected to the output of glucose analyzer. With the software loaded, the computer controls the whole operation of the continuous glucose monitoring system. The data acquisition board consists of 8 channels for differential analog inputs with 12 bit resolution, which accept the analog inputs from 2 mV to 10 V, at a rate of up to 10,000 samples per second [24]. The software has six selectable ranges: from $\pm$ 25 mV to $\pm$ 10 V. It also has an autorange mode, which automatically select a range for the best resolution of any given signal. The data log interval and the number of channels being logged can also be determined by software. At the menu-driven mode, the software allows an investigator, through the IBM computer, to control instrument, acquire data and/or display results.

The data that have been acquired and stored on the floppy disk can be transferred to a application program, such as Lotus 1-2-3 and Sigmaplot, for analysis and graphic display of the data.

Calibration of System

Before samples pumped into the system, the glucose analyzer was first calibrated twice with a glucose standard having 200 mg/dl of glucose. This procedure was repeated for every experiment.

Characterization of System Parameters

For characterization, a series of in vitro studies were performed to systematically investigate various parameters of the system. The effect of variation due to changes in glucose concentration, sample pumping time and speed, diameter of the withdrawing tubing for blood sample, and buffer solution were characterized.

In each study, the system was programmed to have only one parameter varied at each run of triplicate experiments.

Validation of System Accuracy for In Vivo Studies

After selecting an optimal combination of the system parameters for in vivo, the accuracy of the system was first evaluated by pumping buffer solution (which contains no glucose) and glucose standard (200 mg/dl) continuously to check the stability of reading glucose concentration at baseline and glucose standard level.

In order to ensure the capability of the system and the range of glucose concentrations, studies were carried out by continuously pumping a glucose standard into the system. After the readout on the glucose analyzer's display reached the concentration of the glucose standard and was maintained steady for a period of time, buffer solution was continuously added into the glucose standard solution to alter the glucose concentration, while it was measured continuously by the system.

Preparation of Animals

Female New Zealand White rabbits (Davidson's Mill Farm, Jamesburg, New Jersey), weighing 3.5-4.5 Kgs, were fasted overnight. In the following morning, each rabbit was kept in a restrainer cage and its hair around the outer marginal vein (which runs along the outer edge of the dorsal surface of the ear) was shaved by an electric razor. The shaved area was first cleaned with an alcohol swab and lidocaine ointment (5%) was then applied to the area for cannulation to reduce the pain. Vasodilation of the marginal ear vein was attained by local mechanical stimulation. When the vein appeared dilated, a vascular access device with a nonthrombogenic PE-10 tubing (10 inches long) attached was inserted parallel into the vein (the vascular access device was prepared from a Butterfly Intermittent Infusion Set by eliminating the elastic tubing attached to the base of its 21-gauge needle). After gently inserting the PE-10 tubing into the vein (for a depth of $\approx$ 2 cm) through the device, the vascular access device was then removed to leave the PE-10 tubing inside the marginal vein. At the same time, pressure was applied to the insertion site, with a piece of gauze, to stop any bleeding. After the bleeding had been stopped, a piece of surgical adhesive tape was applied over the insertion site to secure the PE-10

tubing. A 0.5 ml of Heparin Lock Flush Solution was flushed, through the PE-10 tubing, into the vein to ensure that no thrombus was formed by this procedure. The procedure was repeated on another ear of the rabbit. After calibration of the system, continuous monitoring of blood glucose in the rabbit was then initiated by connecting a nonthrombogenic blood withdrawing tubing to one of inserted PE-10 tubing for continuous blood withdrawing, using a peristaltic pump.

Determination of Baseline Blood Glucose Level

The blood glucose level in the normal healthy rabbit, without any treatment, was monitored continuously to establish the baseline and to detect any variation in the baseline level of blood glucose resulted from the variation in experimental conditions or biological rhythm.

Hypoglycemic Effect of IV Insulin

Continuous Blood Sampling - The baseline level of blood glucose in each rabbit was continuously monitored. After a stable baseline was established for at least 10 min, the rabbit was given an single intravenous administration of insulin solution (0.05-0.5 ml) and the blood glucose profile was monitored continuously by simultaneous recording on computer and graphic recorder. The insulin solution was prepared by mixing Humulin® R, with the Sterile Water For Injection under an aseptic condition, to obtain a solution with insulin concentration at 1 unit/ml. The administration was carried out by delivering the insulin solution, using a disposable syringe (1 ml), through the PE-10 tubing into the ear marginal vein. Following the administration, the tubing was once more flushing with Heparin Lock Flush Solution. The blood glucose levels were then determined by glucose analyzer continuously for a period of 2 hours and were recorded the result by computer every 20 sec of interval. Same experiment was repeated on three rabbits, and same rabbits were tested again, after a recovery period of at least 2 weeks, with different dosages of insulin.

Intermittent Blood Sampling - To simulate the conventional procedure of blood sampling, the blood samples (1 ml each) were withdrawn through the PE-10 tubing at -3, 3, 6, 9, 12, 15, 20, 30, 45, 60, 90, and 120 min, during the course of continuous blood sampling and monitoring process.

The blood samples withdrawn were each collected into an ice-chilled tube, which contained one drop of heparin (10,000 unit/ml), and was maintained at 2-8°C by immersing in an ice bath throughout the process of blood collection and handling. The glucose level in these blood samples was also determined by the same glucose analyzer immediately as soon as the continuous glucose monitoring experiment is terminate.

RESULTS

Characterization of System Parameters

Effect of Glucose Concentrations - The glucose reading concentrations reach their peak level at the same time for all glucose standards (Figure 2). For short sample pumping time, the peaks appear within 90 sec (Figures 2A, 2B). For sample pumping time sufficient long, the plateau concentrations are attained beyond 90 sec (Figure 2C). Both the peak glucose reading concentration and the area under the glucose reading concentration curve (AUC), which was calculated from trapezoidal rule, increase linearly as increasing the glucose concentration in the standards (Figures 3A, 3C). But, the time to reach the peak is only influenced slightly (Figure 3B), however, it increases as increasing the pumping time of glucose standard. It is interesting to note that the slope of the linearities increases as increasing the pumping time of glucose standards (Figure 3A). It suggests that peak glucose concentration approaches the glucose concentration in the standard as a sample pumping time sufficient long (i.e. 4 min) is used. In other words, the slope value of the linearly increases as increasing the sample pumping time and approaches one at sample pumping time of 4 min. Furthermore, the AUC also increases as increasing the pumping time of glucose standard (Figure 3B).

Effect of Sample Pumping Time - The glucose reading concentration profiles generated from a glucose standard at 200 mg/dl for the various sample pumping times are shown in Figure 4. The glucose reading concentration increases as increasing sample pumping time, and the value of glucose reading concentration reaches the glucose concentration in the standard as the period of sample pumping time is longer than 90 sec. The peak glucose reading concentration increases rapidly at first as the sample pumping time of the glucose standard increases and eventually

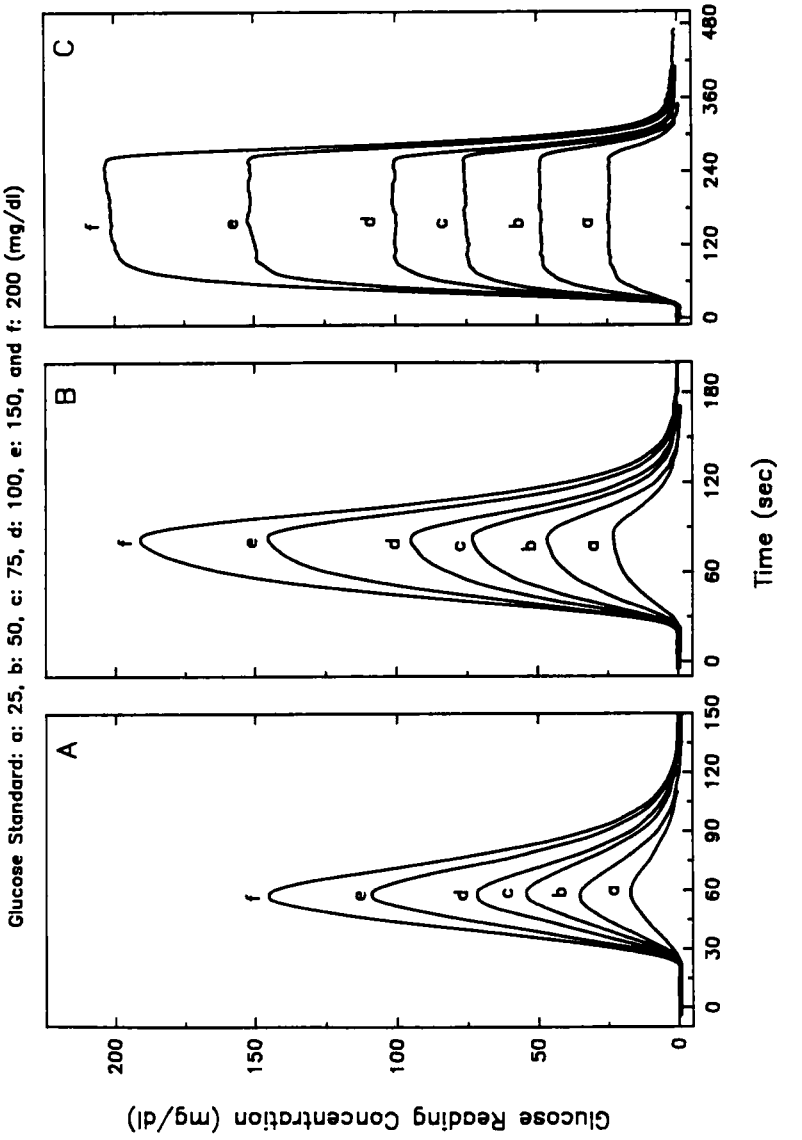

FIGURE 2

Effect of glucose concentration on the glucose reading concentration profiles of glucose standard at various concentration with the sample pumping time of (A) 0.5, (B) 1, and (C) 4 min.

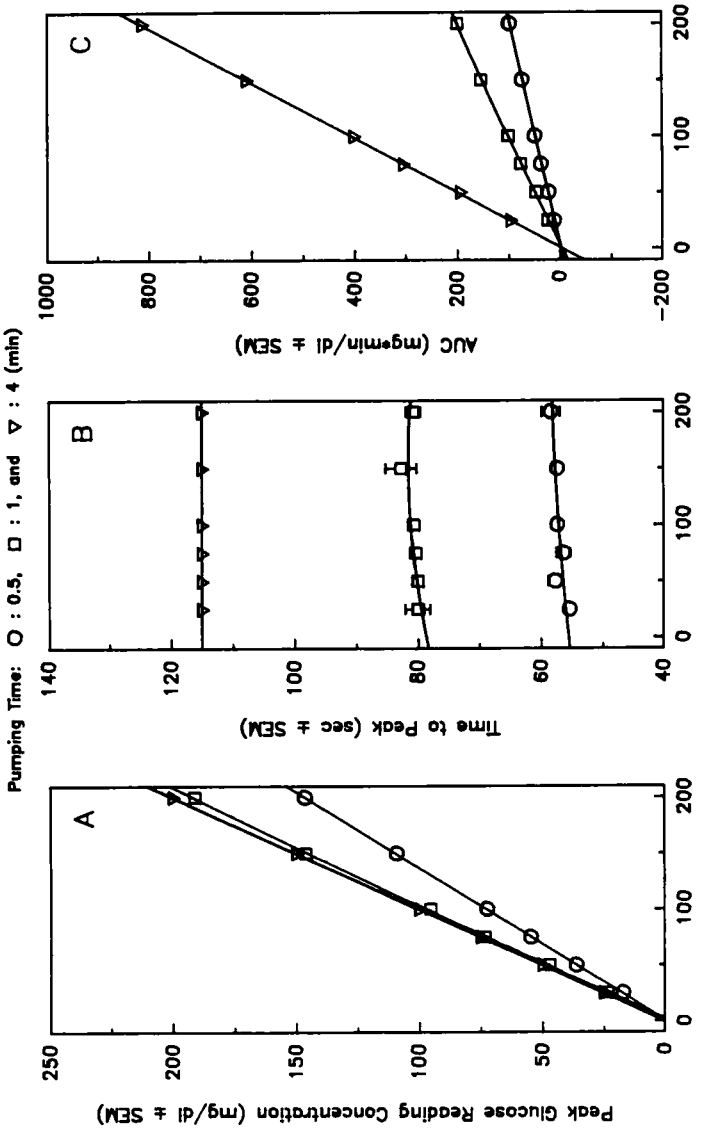

FIGURE 3

The relationship between the glucose standard at various concentrations and the various glucose kinetic parameters: (A) peak glucose reading concentration, (B) time to reach peak glucose reading concentration, and (C) area under glucose reading concentration curve.

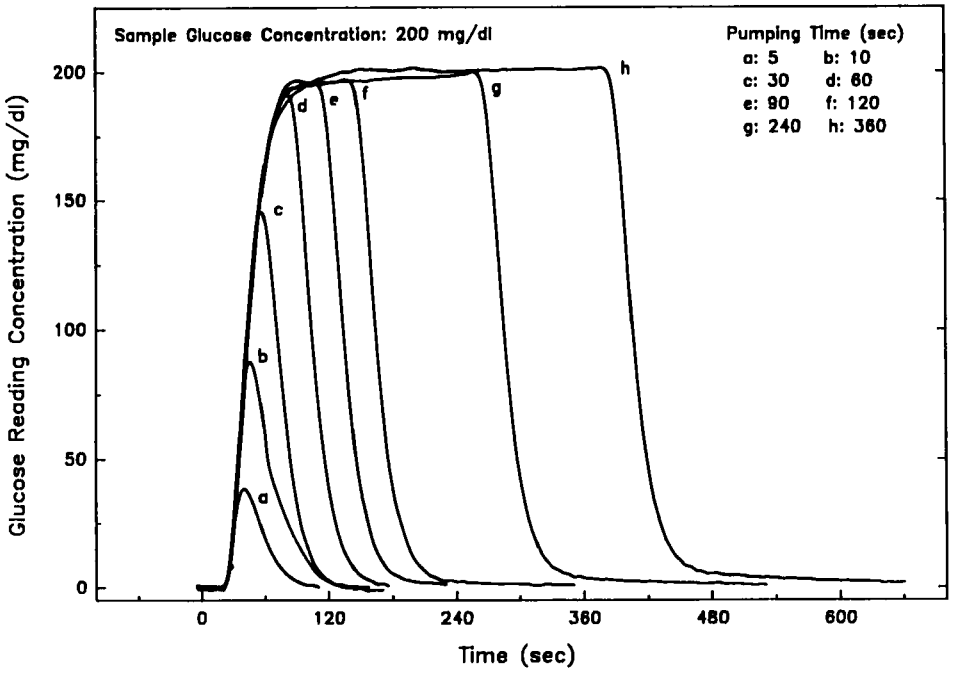

FIGURE 4
Effect of sample pumping time on the glucose reading concentration profiles of glucose standard at 200 mg/dl.

reaches the glucose concentration in the standard within 90 sec (Figure 5A). It suggests that glucose measuring process (i.e. the value of reading glucose concentration is equal to the glucose concentration in the standard), at the system setup used, requires a sample pumping time of approximately 90 sec to complete. The time to peak reading concentration also increases rapidly as the sample pumping time increases, but finally approaches a steady-state level within 110 sec (Figure 5B). Apparently, AUC increases linearly as the sample pumping time increases (Figure 5C).

Effect of Pumping Speed - The higher the pumping speed, the shorter the time required to reach the peak glucose reading concentration and the closer the glucose reading concentration to the glucose concentration in the standard (Figure 6). However, the glucose concentration in the standard cannot be reading when a

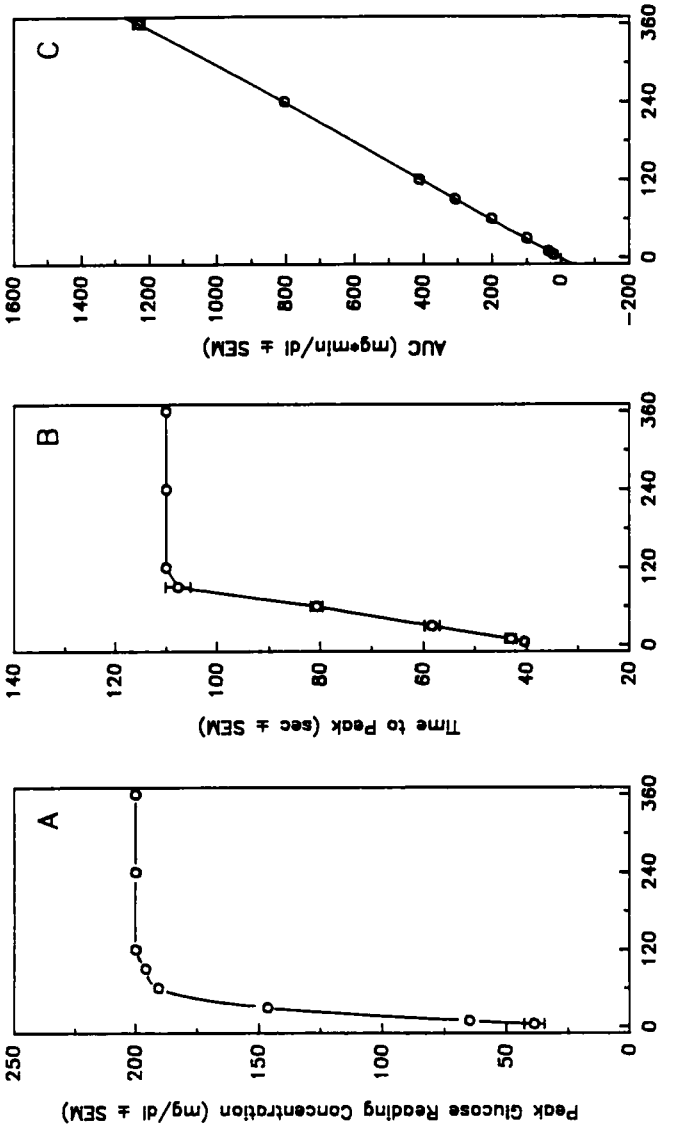

FIGURE 5

The relationship between the sample pumping time of glucose standard (200 mg/dl) and the various glucose kinetic parameters: (A) peak glucose reading concentration, (B) time to reach peak glucose reading concentration, and (C) area under glucose reading concentration curve.

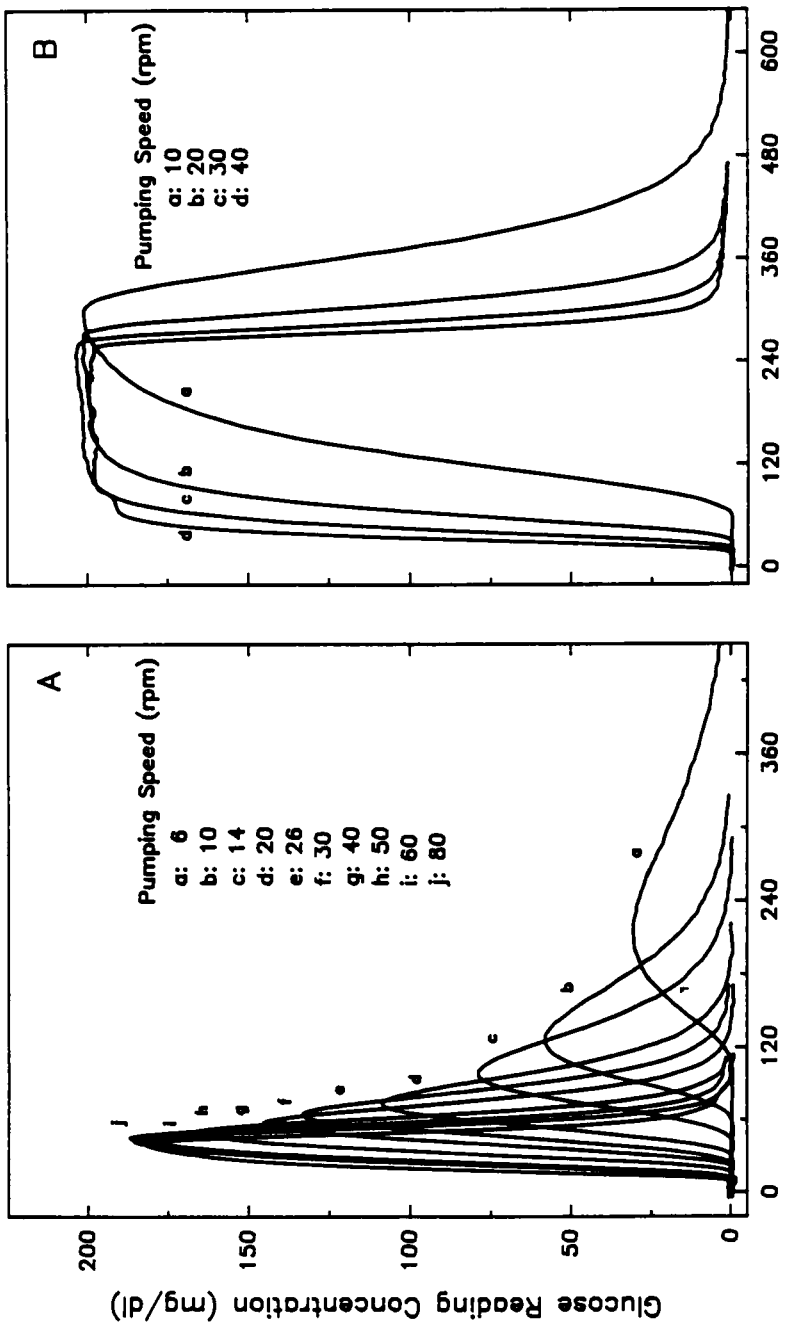

FIGURE 6

Effect of pumping speed on the glucose reading concentration profiles of glucose standard at 200 mg/dl with the sample pumping time of (A) 0.5 and (B) 4 min.

short sample pumping time was used (i.e. 0.5 min). For sample pumping time sufficient long (i.e. 4 min), the glucose standard concentration can be reached at slower pumping speed (i.e. 10 rpm). For short sample pumping time, the peak reading concentration increases as increasing the pumping speed and approaches the steady-state level when the pumping speed is ≥ 50 rpm. However, the agreement between the reading concentration and the concentration in the standard is reached when sample pumping time is increased (Figure 7A). In other words, the glucose measuring process has been completed even at the rate of pumping speed as low as 10 rpm, if sufficient long sample pumping time (i.e. 4 min) is used. The time to reach the peak decreases as increasing the pumping speed and reaches the lowest level at pumping speed ≥ 50 rpm (Figure 7B). The AUC appears to be independent from variations in pumping speeds, but dependent of the sample pumping time (Figure 7C). The value of AUC at sample pumping time of 0.5 min, which is maintained at a constant level of approximately 100 mg/dl•min, is about 1/8 of the 825 mg/dl•min attained at sample pumping time of 4 min. Hence, the AUC is proportionally increased by increasing the pumping time of glucose standard.

Effect of Sample Withdrawing Tubing - The larger the sample withdrawing tubing, the shorter the time required to reach the peak glucose reading concentration (Figure 8). However, the glucose concentration in the standard cannot be reading when a short sample pumping time was used (i.e. 0.5 min). For sample pumping time sufficient long (i.e. 4 min), the glucose standard concentration can be reached a steady-state level at any size of sample withdrawing tubing. Both the peak reading concentration and AUC increase linearly as the square of inside diameter of sample withdrawing tubing increases, and as increasing the pumping time of glucose standard (Figures 9A, 9C). It is interesting to note that the glucose slope of the linearities between peak glucose reading concentration and the square of inside diameter of sample withdrawing tubing is almost the same for both sample pumping times (Figure 9A). The time to reach the peak varies with the variation in inside diameter of sample withdrawing tubing increasing (Figure 9B). For a short sample pumping time (i.e. 0.5 min), the time to reach the peak decreases as increasing the inside diameter of sample withdrawing tubing. For sample pumping time sufficient long (i.e. 4 min), however, the time to reach peak glucose reading concentration does not

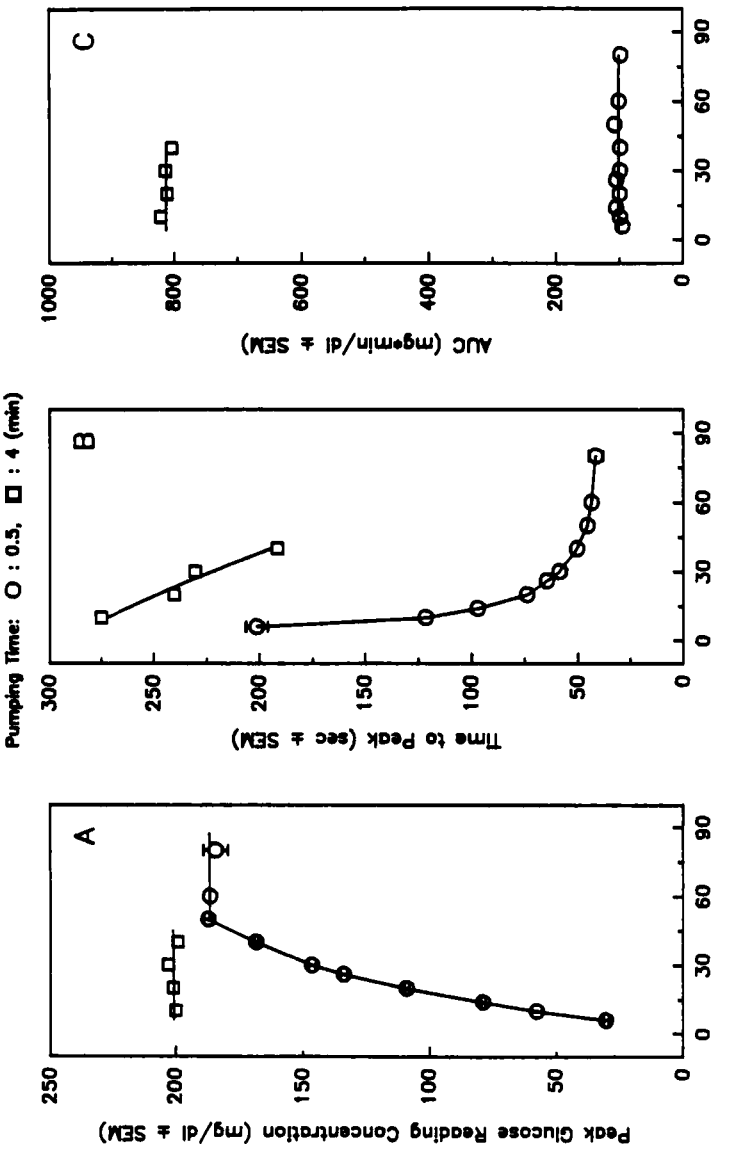

FIGURE 7

The relationship between the pumping speed of glucose standard (200 mg/dl) and the various glucose kinetic parameters: (A) peak glucose reading concentration, (B) time to reach peak glucose reading concentration, and (C) area under glucose reading concentration curve.

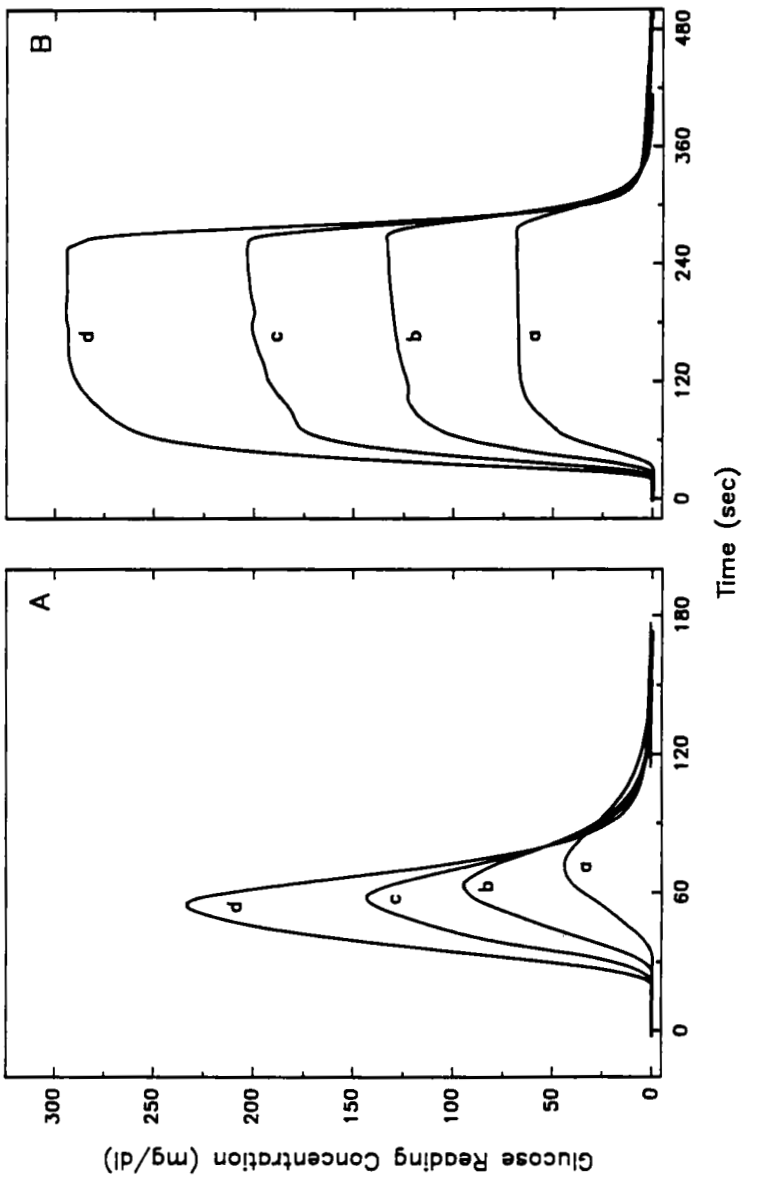

FIGURE 8

Effect of the inside diameter of sample withdrawing tubing on the glucose reading concentration profiles of glucose standard at 200 mg/dl with the sample pumping time of (A) 0.5 and (B) 4 min.

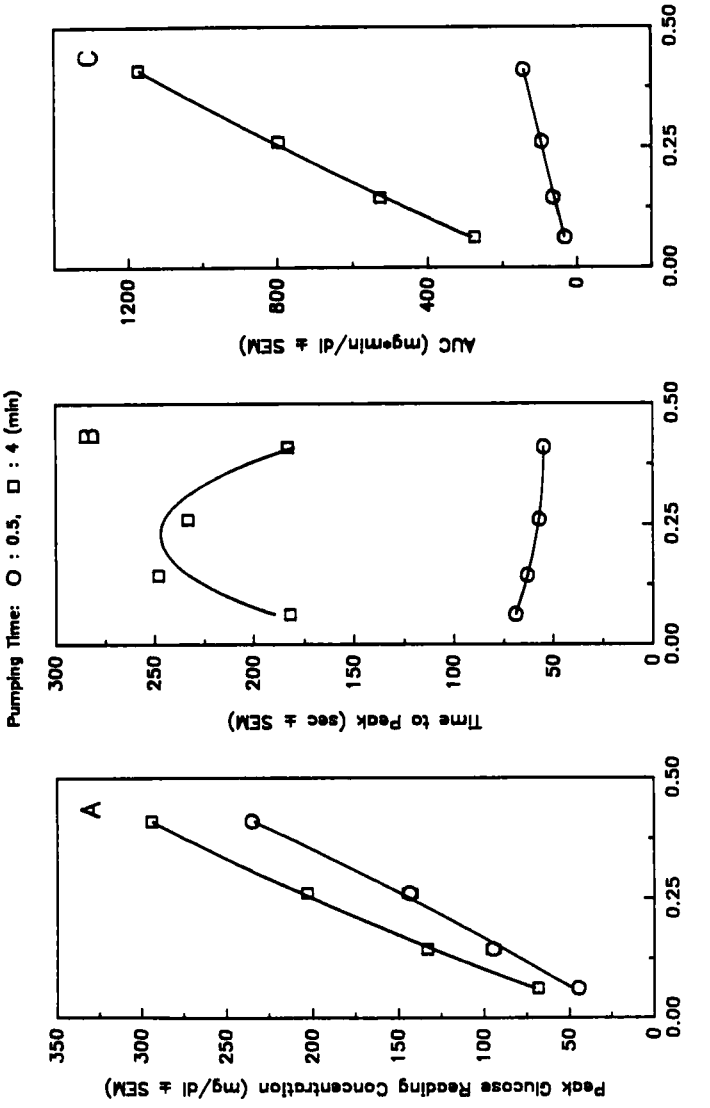

FIGURE 9

The relationship between the square of inside diameter of sample withdrawing tubing of glucose standard (200 mg/dl) and the various glucose Kinetic parameters: (A) peak glucose reading concentration, (B) time to reach peak glucose reading concentration, and (C) area under glucose reading concentration curve.

indicates any meanings, since the glucose measuring process has been completed and reached a steady-state level.

Effect of Buffer Withdrawing Tubing - The larger the buffer withdrawing tubing, the shorter the time required to reach the peak glucose reading concentration (Figure 10). However, the glucose concentration in the standard cannot be reading when a short sample pumping time (i.e. 1 min) was used. For sample pumping time sufficient long (i.e. 6 min), the glucose standard concentration can be reached a steady-state level at any size of buffer withdrawing tubing. The peak reading concentration, the time to the peak, and the AUC values increases linearly as decreasing the inside diameter of buffer withdrawing tubing (Figure 11). The slope of the linearity increases as increasing the duration of sample pumping time.

Validation of System Accuracy for In Vivo Studies

When only buffer solution (containing no glucose) was pumped into the continuous glucose monitoring system, once calibrated with a glucose standard, the glucose concentration shown on the recording chart and the digital display was found to be at baseline level (zero), as expected (Figure 12A). In Figure 12A, the rapidity and the accuracy of the system in measuring glucose concentration in response to the alternatively pumping of buffer solution and glucose standard (200 mg/dl) into the system are also shown. The good correlation between the glucose concentration detected by the system and actual glucose concentration in the standard pumped into the system was also observed and the stability of the measurement was demonstrated throughout the course of 7-hr continuous pumping of glucose standard with the system operated continuously without interruption (Figure 12B). In Figure 12C, it indicated that the glucose concentration detected by the system was also complied with the theoretic value perfectly. However, the correlation appears badly when the glucose value below 25 mg/dl. The results of validation study performed in this investigation demonstrated that the continuous glucose monitoring system developed is accurate and reliable.

Determination of Baseline Blood Glucose Level

The blood glucose concentration in the healthy rabbit without any medication was also measured by the continuous glucose monitoring system following calibration

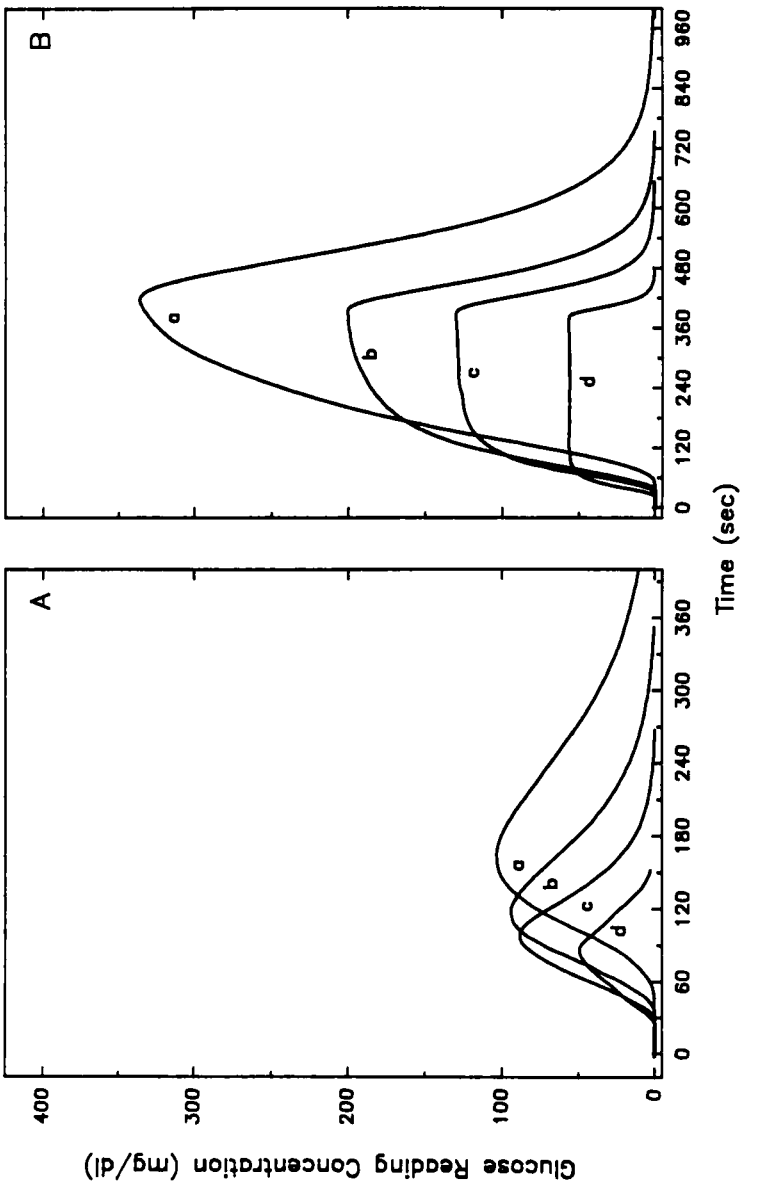

FIGURE 10

Effect of the inside diameter of buffer withdrawing tubing on the glucose reading concentration profiles of glucose standard at 200 mg/dl with the sample pumping time (A) 1 and (B) 6 min.

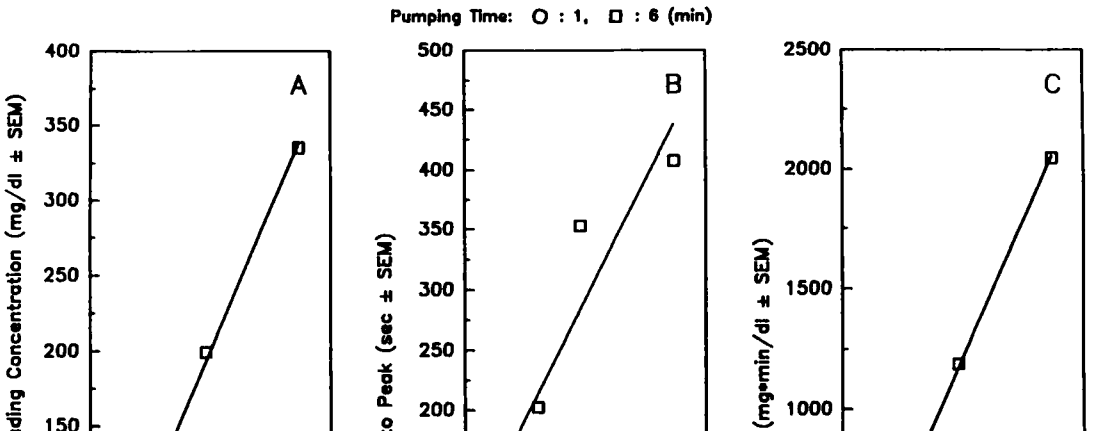

FIGURE 11

The relationship between the reciprocal of the square of inside diameter of buffer withdrawing tubing of glucose standard (200 mg/dl) and the various glucose kinetic parameters: (A) peak glucose reading concentration, (B) time to reach peak glucose reading concentration, and (C) area under glucose reading concentration curve.

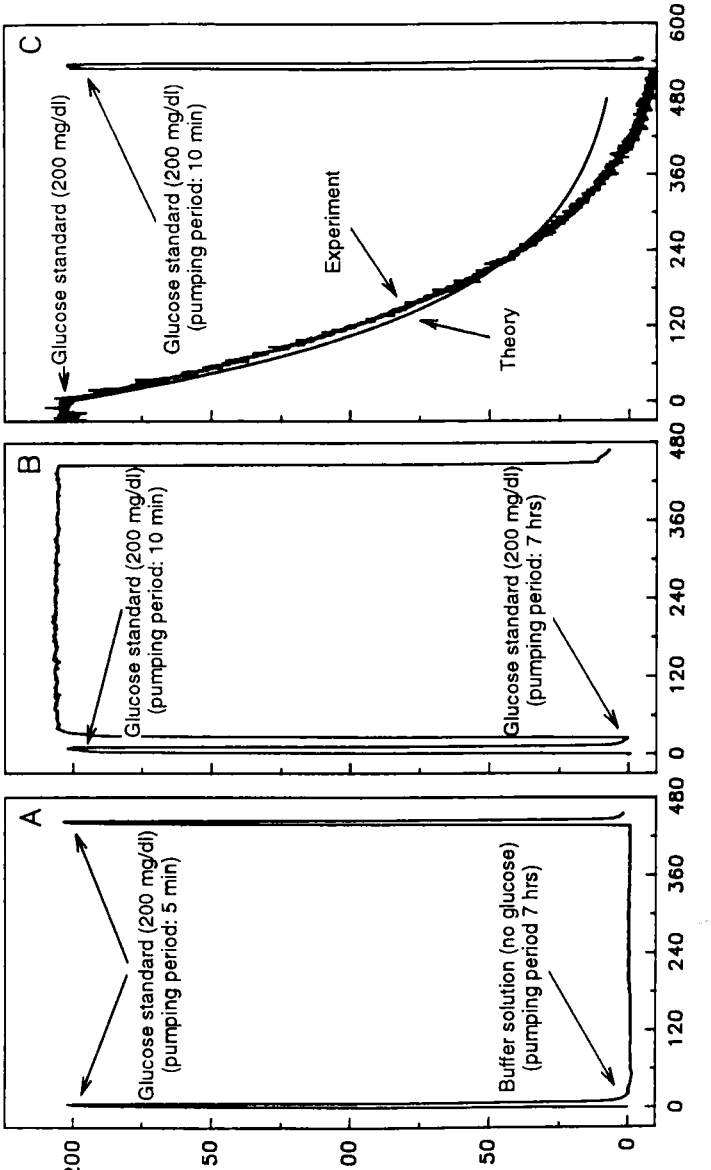

FIGURE 12

The glucose reading concentration profiles following the pumping of (A) buffer solution (containing no glucose), (B) glucose standard (200 mg/dl), and (C) various glucose standard concentrations (0-200 mg/dl) into the continuous glucose monitoring system after the system had been calibrated with a glucose standard (200 mg/dl).

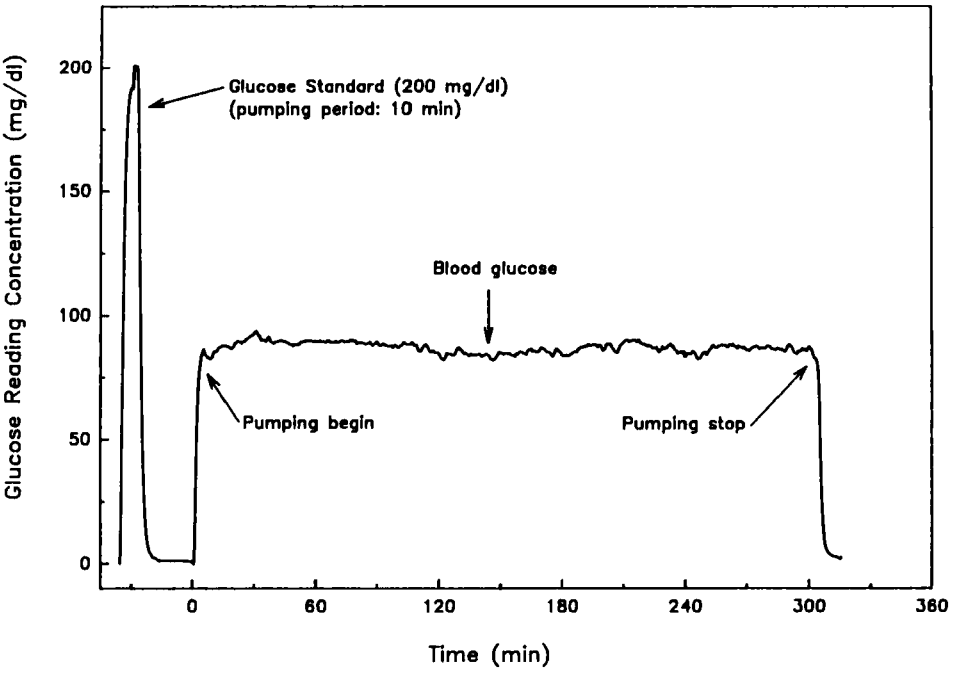

FIGURE 13
The glucose concentration profile in a healthy rabbit (without medication) measured by the continuous glucose monitoring system which had been precalibrated with a glucose standard (200 mg/dl).

of the system with glucose standard (200 mg/dl). A constant baseline level of 84 mg/dl was detected, which was maintained at a reasonable (± 5%) steady state throughout the course of at least 5 hours (Figure 13).

Hypoglycemic Effect of IV Insulin

A typical profile for the reduction of blood glucose level from the baseline following the intravenous administration of 0.5 ml insulin solution (0.5 IU/rabbit), as measured by both the continuous and intermittent blood sampling techniques, is graphically shown in Figure 14. The results indicate that the hypoglycemic response profile measured by the continuous glucose monitoring system developed in this investigation is very much identical to that attained by the conventional, time-consuming intermittent blood sampling techniques. In other words, the hypoglycemic

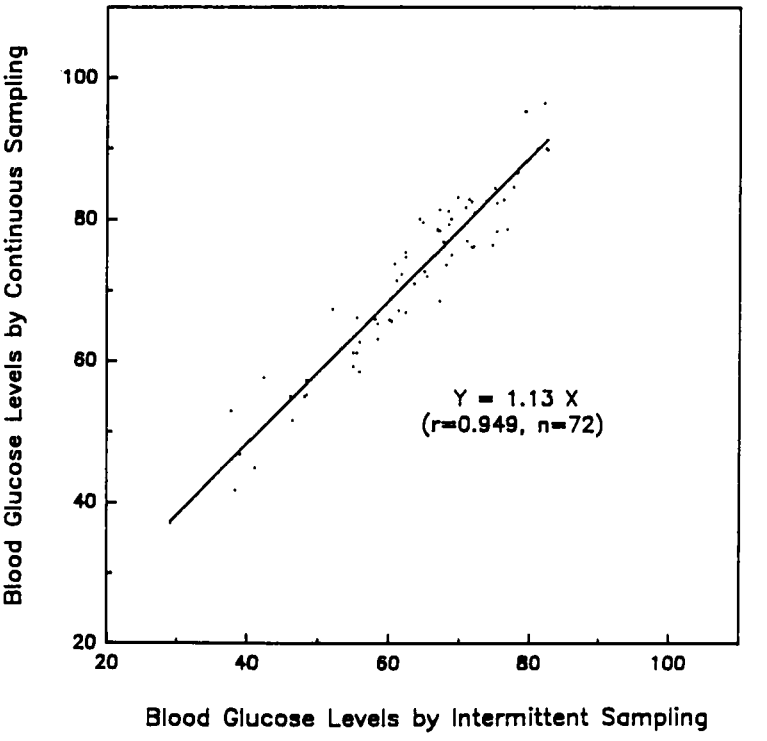

FIGURE 14
A typical set of results on the reduction of blood glucose level from the baseline, as measured by continuous and intermittent blood sampling methods in the same rabbit, following the intravenous administration of 0.5 ml insulin solution (0.5 IU/rabbit).

effects attained by both techniques were compared and analyzed statistically and the results indicated that no significant difference could be detected. Considering the individual glucose determination, the comparison of glucose values by both methods was plotted in Figure 15. The blood glucose levels determined by continuous sampling is linearly correlated with those by intermittent sampling ($r = 0.95$) with a slope value of 1.13.

DISCUSSION

In the previous glucose monitoring systems, a double lumen intravenous catheter was used for blood withdrawing [6-10]. A heparin solution is pumped through one of the double lumens to the tip of the catheter inserted into a vein. The

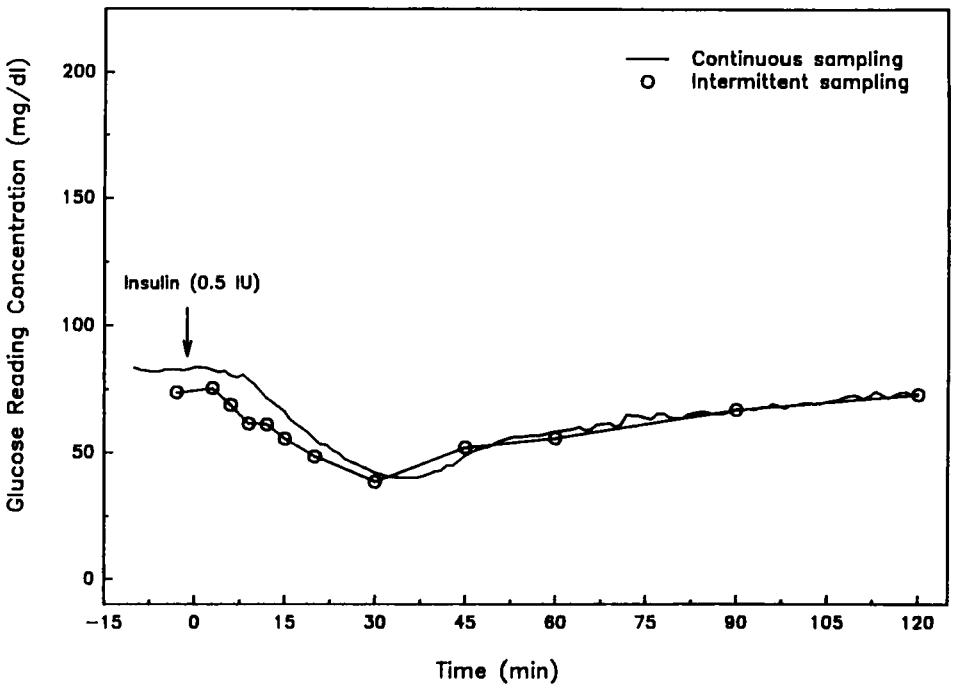

FIGURE 15
Comparison of blood glucose concentrations obtained by the intermittent sampling method with those obtained by continuous sampling method.

mixture of blood and heparin is withdrawn through the second lumen. This could create some technical problems since the interruption of blood flow can not be easily detected. Using this system, any interruption of the blood flow through the nonthrombogenic tubing, which is transparent, can be easily noticed.

In this study, an optimal combination of the pumping speed, the inside diameter of withdrawing tubing for blood sample and buffer solution are (10 rpm, 0.25, and 1.02 mm, respectively) were used. With this setup, the blood was withdrawn from animal at the pump rate of 2-3 ml/hr, while it requires 30 ml/hr with the double lumen intravenous catheter [6-10]. The lag time of around 2 min, from blood withdrawal to glucose measurement, was attained. In other words, the result of the glucose measurement can be obtained at 2 min after the withdrawal of blood sample from the animal. It was necessary to initiate the continuous

measurement of blood glucose until the stable baseline level has been maintained for at least 10 min. Thereafter, any change of glucose concentration can be detected accurately and continuously.

By assuming the initial baseline level of blood glucose measured in each rabbit as the 100% level for that rabbit, one can estimate the change in blood glucose level at any time following an insulin treatment by dividing the glucose concentrations after the treatment by the initial baseline before the treatment. By such approach, the effect of insulin treatment on blood glucose profiles in a group of rabbits can be analyzed together statistically without the influence of intrinsic inter-animal variability. Therefore, for the purpose of analyzing the hypoglycemic effect of insulin, the following three pharmacodynamic parameters have been established:

1) Nadir: the lowest level of blood glucose, expressed in % of the initial baseline, attained by an insulin treatment.

2) Time to Nadir: the time to reach the nadir.

3) ABGC: the area of the blood glucose response curve under the baseline.

The pharmacodynamic parameters for the hypoglycemic effect of insulin determined by the continuous and intermittent blood sampling techniques are compared. Statistical analysis (pair t test) indicates that there is no significant difference between these two sets of data ($p > 0.05$), which suggests that the results obtained by this newly-developed continuous glucose monitoring system, in this study, are comparable to the data generated by the conventional intermittent sampling method. It could be significant deference, however, when the nadir does not occur at the predetermined intervals of intermittent sampling.

The dose-dependence of these pharmacodynamic parameters are shown in Figure 16. While the values of nadir and the time to nadir show nonlinear dependence on the intravenous dose of insulin (Figures 16A, 16B) for both sampling techniques, it is encouraging to observe a linear relationship exists between the ABGC values determined by continuous glucose monitoring technique and the dose of insulin administered (Figure 16C). On the other hand, no linear relationship can be established for the conventional intermittent sampling technique. This

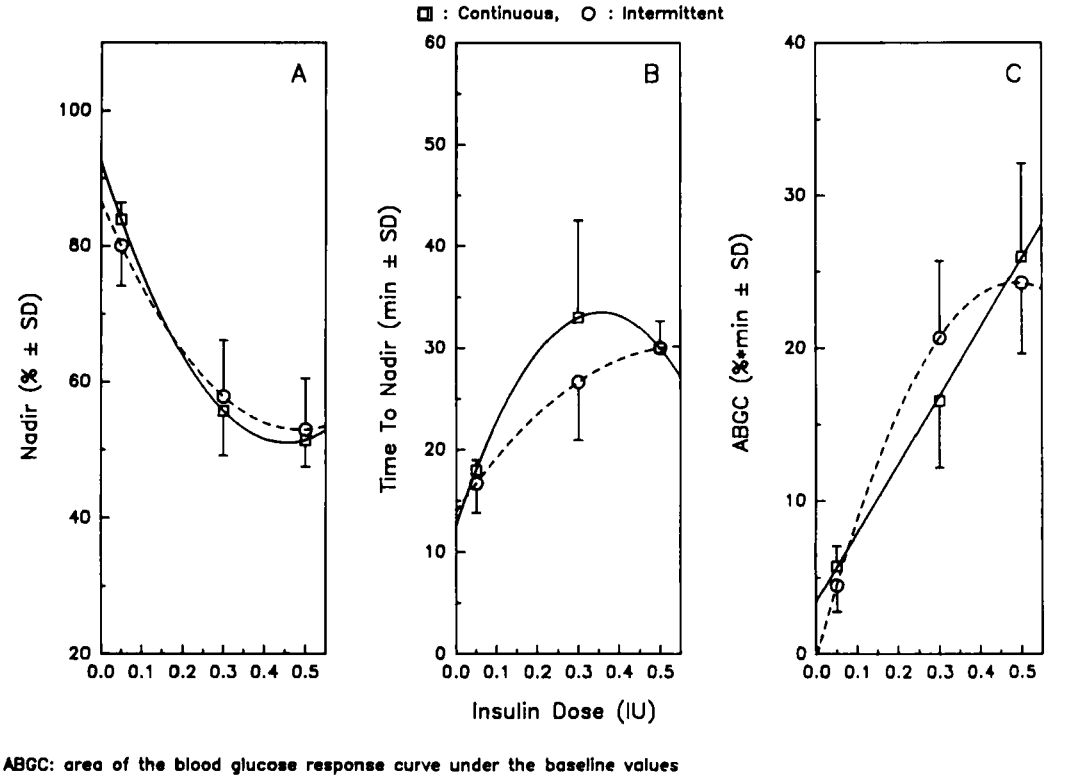

FIGURE 16
The relationship between the insulin dose administered intravenously and the various pharmacodynamic parameters: (A) nadir, (B) the time to nadir, and (C) the area of blood glucose response curve (ABGC) determined from the blood glucose response profiles assayed by the continuous and intermittent blood sampling methods.

observation suggests that the ABGC can be used as a valid parameter for the prediction of hypoglycemic activity for a given insulin dose or for the bioassay of insulin potency in a pancreas extract or biotech product.

CONCLUSIONS

A continuous glucose monitoring system developed, under the cost of 10,000, provides an accurate means of monitoring glucose concentrations, on a continuous on-line basis, in the conscious animals without any interruption and/or disturbance. Furthermore, a continuous blood sampling technique was successfully developed for

continuous glucose monitoring in the rabbit without surgery and/or anesthesia. This enables an accurate determination of the peak of glucose concentrations, even though it lasted for only a few seconds and occurred at unpredictable moment. Meanwhile, it has the potential to achieve a great saving in labor and time needed to conduct an experiment as well as in the number of animals required for the desired accuracy.

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