Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biosensors and Bioelectronics 18 (2003) 899–905
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

# A microdialysis technique for continuous subcutaneous glucose monitoring in diabetic patients (part 2)

M. Varalli [a], G. Marelli [b], A. Maran [c], S. Bistoni [f], M. Luzzana [e], P. Cremonesi [e], G. Caramenti [d], F. Valgimigli [a], Alessandro Poscia [a,*]

[a] *Research and Development Department, A. Menarini Industrie Farmaceutiche Riunite S.r.l., Via Sette Santi, 3-50131 Florence, Italy*
[b] *Divisione di Medicina-Servizio di diabetologia, A.O. Presidio Ospedaliero di Desio, Piazza Benefattori, 20033 Desio, Milan, Italy*
[c] *Dipartimento di Medicina Clinica e Sperimentale, Malattie del Metabolismo, Università di Padova, Via Giustiniani, 2-35128 Padua, Italy*
[d] *I.T.B. Istituto di Tecnologie Biomediche, C.N.R. Via Fratelli Cervi, 93-20090 Segrate, Milan, Italy*
[e] *Dipartimento di Scienze e Tecnologie Biomediche, Università di Milano, Via Fratelli Cervi, 93-20090 Segrate, Milan, Italy*
[f] *Dipartimento di Medicina Interna e Scienze Endocrine e Metaboliche, Università di Perugia, Via Enrico Dal Pozzo, 06126 Perugia, Italy*

Received 23 March 2001; received in revised form 15 August 2001; accepted 19 August 2002

**Abstract**

The aim of this study was to evaluate the reproducibility, the accuracy and the reliability of a continuous subcutaneous glucose measuring system. The GlucoDay® system (A. Menarini I.F.R. S.r.l.—Florence, Italy) is a portable instrument provided with a micro-pump and a biosensor, coupled to a microdialysis system (see part 1). This instrument has demonstrated high reliability coupled with a low degree of invasivity. The profiles of glucose monitoring allow to achieve an excellent knowledge of the real variation of glucose in diabetic patients. The reproducibility study showed a bias lower than 10% between instruments. The accuracy study showed a difference from the reference method lower than 15%.


*Keywords:* Biosensors; Continuous monitoring; Glucose; Microdialysis

## 1. Introduction

Intensive treatment and close diabetes mellitus monitoring can prevent progression of long term complications in the diabetes mellitus disease (The DCCT Research Group, 1993). Accurate and systematic blood glucose monitoring is a key element to prevent development of complications. For this reason, in recent years, a number of systems capable of measuring the blood glucose level in a very accurate and simple way have been developed (Turner et al., 1980). This allows patients to keep their blood glucose levels under control everywhere at anytime and it provides the physician with a more correct and detailed picture of the disease (Clarke et al., 1987).

However, a monitoring performed according to these new systems does not allow an overall, clear and in-depth view of the actual glycaemic situation. In fact, in some subjects, namely type 1 patients, glycaemia may undergo rapid changes and the discrete monitoring measurements, performed by the usual blood glucose self-monitoring devices, is not able to ensure an actual and effective control.

Moreover, the procedure, which requires a great number of capillary blood samples, causes patient's discomfort.

On the other hands, a continuous and fully automatised glucose monitoring device represents an alternative to frequent capillary blood collection. In fact, the patient does not have to perform any measurement, which are instead automatically and independently taken by the device, even during work/rest hours. This rends the study and the control of glycaemia easier and more accurate for the physician.

* Corresponding author. Tel.: +39-055-5680-368; fax: +39-055-5680-576
*E-mail address:* aposcia@menarini.it (A. Poscia).

0956-5663/02/$ - see front matter 
PII: S0956-5663(02)00215-4

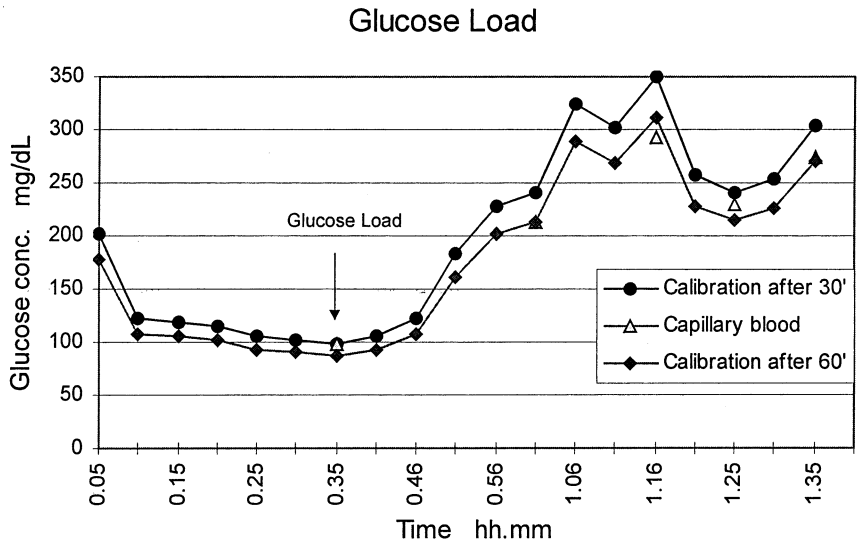



Fig. 1. Glucose continuous monitoring during a glucose load.

Continuous monitoring of glucose (the most important metabolite in diabetes care) was introduced several years ago with a complex instrument called the biostator (Fogt et al., 1978). Other methods employing the enzyme glucose oxidase coupled to an electrochemical transducer have been developed throughout the years (Pickup et al., 1989). These devices have been coupled to a microdialysis system (Meyer et al., 1993; Pfeiffer et al., 1993) or have been hypodermically implanted (Claremont et al., 1986; Koudelka et al., 1991; Pickup, 1993; Shaw et al., 1991).

The new generation of biosensers must meet requirements such as, high sensitivity, low power requirement, extremely fast response, not frequent calibration and miniaturisability (Pickup and Alcock, 1991). An example is represented by the FDA approved device (Minimed), which measures the glucose directly in the interstitial fluid every 3–5 min without lancets. However, it presents the disadvantage of a not good relation with the venous glucose values below 50 mg/dl (Johnson et al., 1992). During this year, another device for continuous monitoring of glucose (GlucoWatch) has been approved by FDA. It collects data every 20 min. In addition, it is possible to calibrate the instrument after 3 h of warm-up (Garg et al., 2002a,b).

In this paper we present a new system, for in vivo glucose continuous monitoring developed and produced by A. Menarini Industrie Farmaceutiche Riunite s.r.l. (Florence, Italy). It is based on the micro dialysis technique and it is minimally invasive.

After preliminary tests (see Part 1), called feasibility study, in which the system showed a very satisfactory performance, in this paper we report the results obtained during in vivo experiments on human volunteers.

## 2. Materials and methods

### 2.1. The apparatus

The characteristics of the device (the programmable micropump, the fluidic line, the wall jet flow cell, the RS232 and Infra Red interface, and the microcontroller) are described in the previous article (see Part 1).

### 2.2. The glucose sensor

The wall jet flow cell, including the electrode, is made by a thin membrane of cellulose acetate (20 μm), a nylon net (thickness 100 μm) in which the enzyme glucose oxidase is immobilised and a polycarbonate membrane (see Part 1).

### 2.3. The microdialysis probe

A microdialysis fiber, with opposite inlets (as shown in article part 1) is connected to the system with nylon tubes and it has been used for in vivo experiments on human volunteer. The exposed length of the fiber is 2.5 cm, however, only 1.5–2 cm are inserted under the skin.

### 2.4. Chemicals

The perfusion solution was prepared by adding 1 g/l of a preservant (such as sodium benzoate) to a Dulbecco's physiological buffer (NaCl 136.9 mM, KCl 2.7 mM, $KH_2PO_4$ 1.5 mM, $Na_2HPO_4$ 8.1 mM, pH 7.4).

The glucose calibration solution (0.5 mM) was prepared by dissolving β-D(+)glucose (Sigma) and $NaN_3$ into a Dulbecco's physiological buffer, and it was used for the sensor check before the in vivo monitoring.

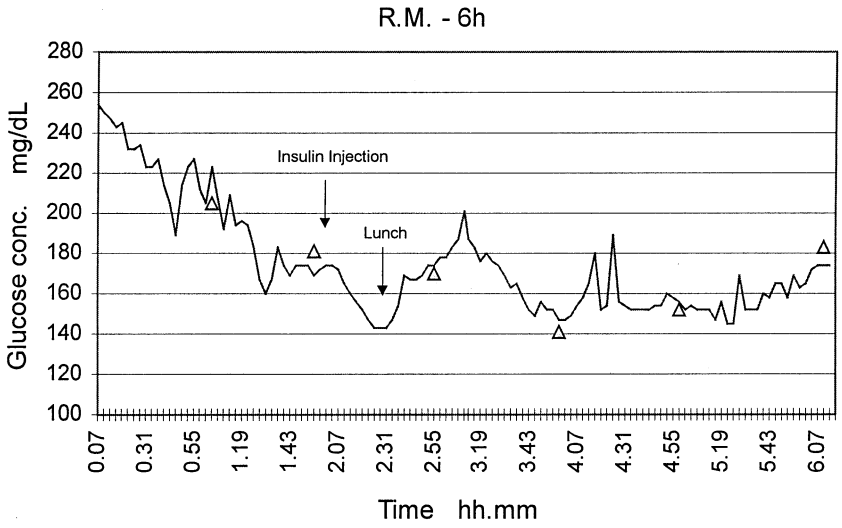



Fig. 2. Six hours of continuous monitoring of a diabetic patient type 1.

### 2.5. Procedures

The system was checked by in vivo experiments on human volunteers using sterilised microdialysis fibers inserted subcutaneously through the skin. The microdialysis fiber, not yet connected to the flow system, was inserted in the periumbilical region, an area with a good vascularisation and little smooth muscle, to avoid any involuntary muscle contractions, that could collapse the fibers. A local anesthetic may be applied before the insertion. The cannula needle (Insyte 18G) was inserted beneath the skin, previously disinfected, piercing the skin and taking the needle parallel to the skin. Once the fiber reached the correct position in the Teflon guide and in the connector, the nylon tubes were connected to the instruments and the physiological solution was pumped through the system. After controlling that the solution was flowing without leaks or obstructions, the Teflon guide and the connector were removed carefully leaving the microfibers in place. Finally, it was fixed using surgical tapes. This procedure took between 5 and 10 min. The instruments were then fully activated allowing the perfusion solution to flow into the micro dialysis fibers. The volunteer's blood glucose values were recorded by the instruments every 3 min.

After 30–60 min, which are normally sufficient for stabilisation of glucose concentration in the perfusion solution, in vivo calibration of the instruments was performed. This was done by testing the venous blood glucose level (checked by Beckman, Ohio, USA) and matching the signal (current value) from the instruments.

During the experiments the values of subcutaneous glucose concentrations were compared with blood samples drawn from vein or finger and the glucose signal recorded by the instruments.

### 2.6. Human volunteer

We tested the device inserting one sterilised micro dialysis fiber in a human volunteer (male, healthy volunteer) under a glucose loading, in order to exactly assess when to perform the calibration (see Fig. 1). Informed consent was obtained from all the subjects.

Further evaluation was performed in order to optimise the calibration. In fact we reported another experiment in a diabetic patient (see Fig. 2) where the glucose concentration was monitored for 6 h using two different points of calibration. The result was the same as in the previous experiment.

We repeated the experiment with another patient and we checked our system not only with capillary blood but also with a venous blood sample (glucose in venous plasma checked with Beckman instrument). We wanted to assess whether our system was better correlated to capillary sample rather than venous sample. Then we extended the monitoring up to 24 h on a diabetic patient to understand if the microfiber was able to dialyse for such a long time, if the sensor was able to maintain the same calibration coefficient until the end of the experiment and finally if the whole system was able to monitor in continuous for 24 h.

In order to study the reproducibility of the system we inserted two different micro fibers in the same patient. Both fibers were inserted subcutaneously in the same periumbilical region, and the instruments were placed on each side (left and right). We calibrated the instruments with a venous sample, taken after 2 h and checked the monitoring with capillary sample by fingerstick. After 24 h we performed again a venous check (see Fig. 5 panel A–C). After the initial calibration, the instruments were synchronised. At the end we performed a 48 h continuous glucose monitoring, checking with capil-

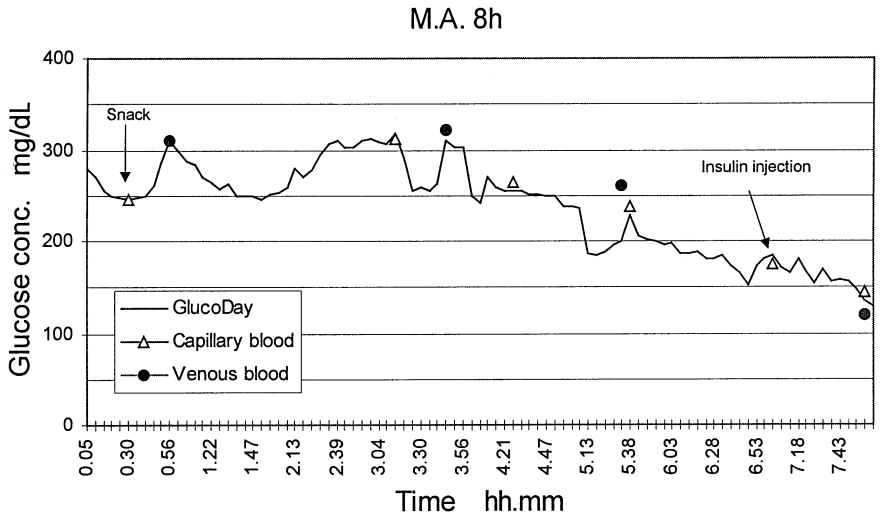



Fig. 3. Continuous monitoring compared with capillary and venous blood sample.

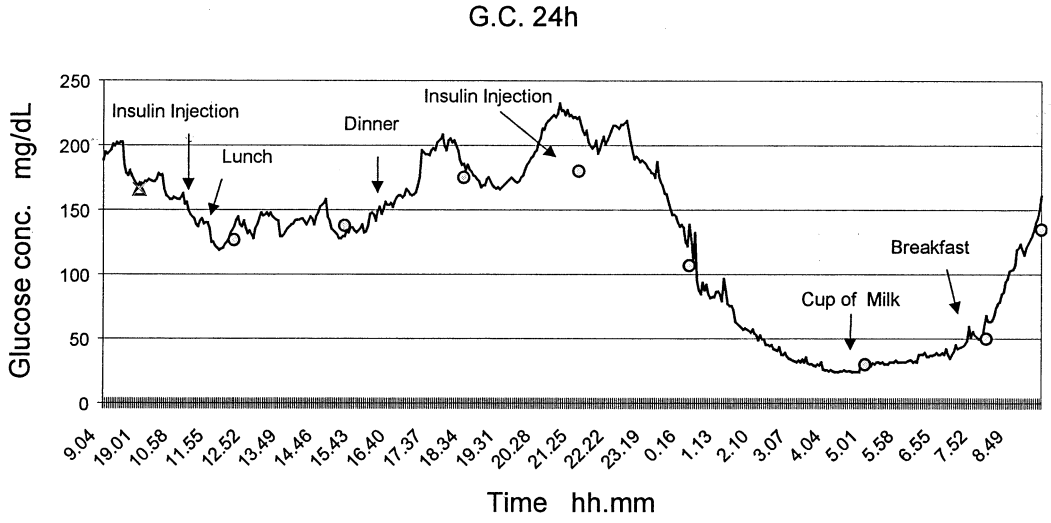


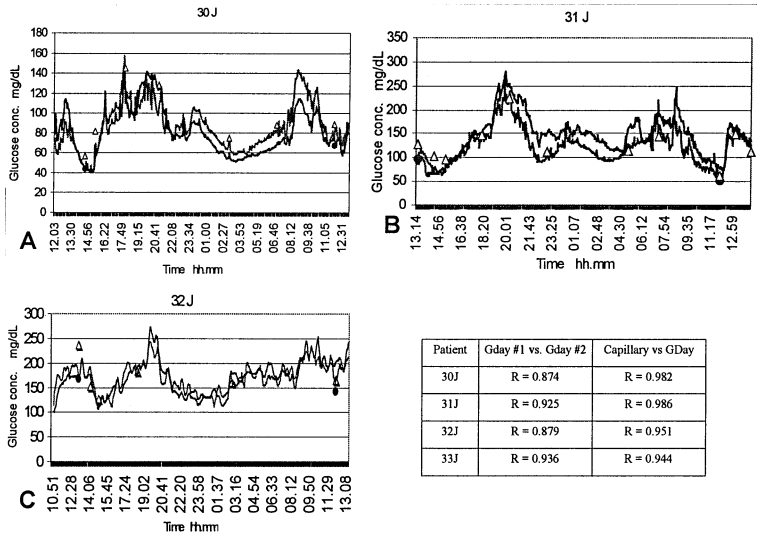

Fig. 4. Twenty-four hours of continuous monitoring of a diabetic patient (type 1). It is very interesting to see a severe nocturnal hypoglycaemia event and then a very quick increase in the early morning.

lary blood samples (see Fig. 6) and with venous blood samples (see Fig. 7).

## 3. Results

Fig. 1, shows the profile of glucose concentration in a healthy volunteer during a glucose load. In the graph two different profiles monitored by GlucoDay® are shown; one with a calibration performed 30 min after the micro fiber insertion and the other with a calibration performed 60 min after the micro fiber insertion. In the second case the system was more accurate, with an absolute mean bias of −5 mg/dl (−3.6%) for the whole experiment.

In Fig. 2, the glucose profile of a diabetic patient monitored for 6 h is shown. In case of a monitoring with a calibration performed 60 min after the micro fiber insertion we obtained the best accuracy of the system (absolute mean bias −2.4 instead of 14.4 mg/dl). This result can account for two different reasons: one is due to the monitoring system and the other is related to the physiology. In fact, if some times it is necessary to reach the equilibrium with the micro dialysis under the skin, it is likewise true that it is necessary to reach the physiological omeostasis under the skin after the needle insertion (stress condition and release of cortisol, adrenaline etc.). These substances stimulate the liver to grant the glucose release. The best solution is to postpone the calibration 1 h after the micro fiber insertion.




| Patient | Gday #1 vs. Gday #2 | Capillary vs GDay |
|---|---|---|
| 30J | R = 0.874 | R = 0.982 |
| 31J | R = 0.925 | R = 0.986 |
| 32J | R = 0.879 | R = 0.951 |
| 33J | R = 0.936 | R = 0.944 |

Fig. 5. Twenty-four hours of continuous monitoring with two different instrument on the same patient. Patient 30J, 31J and 32J (33J not showed) present an excellent reproducibility and a very good accuracy for both blood samples (○, venous; △, capillary).

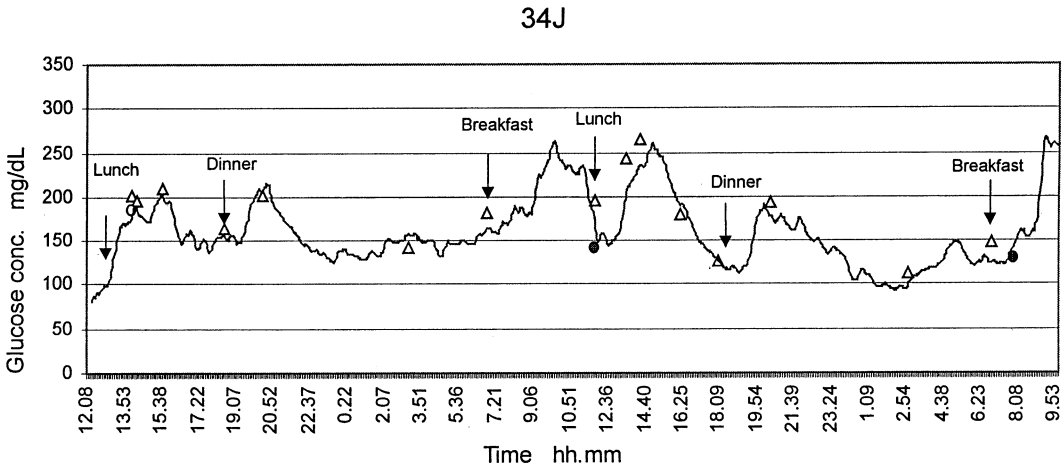



Fig. 6. Forty-eight hours continuous monitoring diabetic patient type 2. Line: GlucoDay; ○, venous blood sample; △, capillary blood sample.

Fig. 3 shows the correlation with two different kinds of blood: capillary and venous blood. In the first case we obtained an average bias percentage of −1.3%, in the second case we obtained −3.6%.

Fig. 4 reports the 24 h experiment, showing that GlucoDay® is able to provide an accurate glucose profile for the all experiment duration. From the data obtained during the experiments, we can observe that GlucoDay® shows an increasing overestimation during the 24 h monitoring. During the day period we observed a constant increase of the percentage bias up to +30% at end of experiment. This confirms that the micro fiber worked properly for all the time and that the overestimation was correlated to the biosensor or to the system. We have data (not showed) explaining that this drift is due to the micro dialysis system and not to the biosensor. Actually, on the basis of experiments, we exclude that the drift problem depends on the biosensor (because in this case the drift would have been negative: loss of current signal). The reason why we had a positive drift was due to the loss of power of the battery of the micro pump, which during the experiment, delivered the perfusional liquid at constant rate only until the battery voltage was constant. However, the discharge of the battery was not linear and the micro pump worked with a rate not constant along the monitoring period. During the experiment the pump slowed down so that, at the end, the micro dialysis was more concentrated than at the beginning. The increase of micro dialysis is proportional to the decrease of the battery charge. For this reason we implemented into the system a compensation in order to correct this drift. This was achieved by

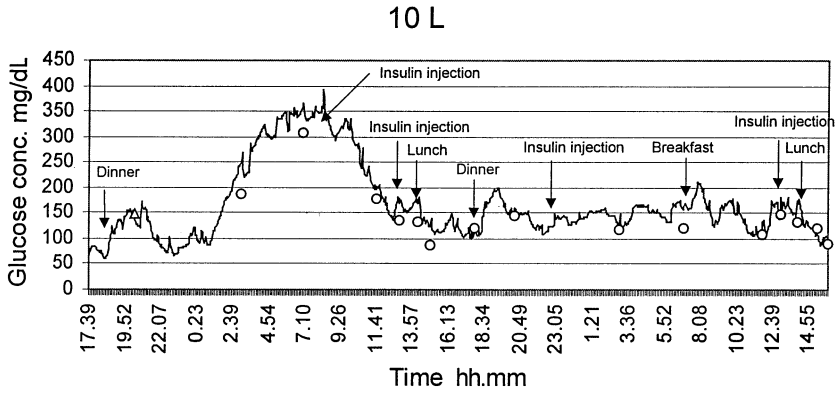



Fig. 7. Forty-eight hours continuous monitoring diabetic patient type 1. Line: GlucoDay: ○, venous blood sample.

modifying the firmware, which is now able to control the rate of the pump, maintaining it constant for at least 50 h.

In Fig. 5 (panel A–C) we present three different 24 h continuous monitoring performed with two different instruments on the same patient in order to study the reproducibility of the system. We obtained the results presented in the table and inserted in the Fig. 5. A linear equation of $y = 1.0657x - 5.323$ and a regression coefficient of $r = 0.9614$ was obtained between all capillary blood samples and all GlucoDay® instruments. These experiments proved several points: the instruments, after calibration, are practically overlapping (mean correlation coefficent: $r = 0.9034$); particular glycaemia shapes are probably consistent (short peaks); the micro fiber insertion point is the optimal.

Fig. 6 shows a 48 h continuous monitoring of glucose performed with a single venous blood calibration (after 2 h), a venous check (after 24 h) and another venous check (after 48 h). Between these checks we performed 15 different glucose measurements in capillary blood. We obtained a good correlation with capillary samples ($r = 0.9097$). This result is very interesting because it was obtained with only one calibration point performed 2 h after the micro fiber insertion. Percentage bias from venous blood samples are less than 15%.

Fig. 7 shows a 48 h continuous monitoring of a diabetic patient (type 1) performed with one venous blood calibration (after 2 h) and with 17 different glucose measurements in venous plasma. We obtained a very good correlation with venous samples ($r = 0.9567$), with an overall bias percentage within 15%. As in the previous experiment, we obtained good results with one calibration point up to 48 h of continuous glucose monitoring.

## 4. Conclusions

The instrument composed by the glucose biosensor, with good performance, and the micro dialysis probe, could give a significant contribution to the treatment of the diabetes. Only continuous glucose monitoring over the 24–48 h period is the key to reach an appropriate metabolic control in order to avoid both the long-term consequences of diabetes and the immediate danger of hypoglycaemia. In fact the finger sticking method shows only a poor approximation of the blood glucose variations, especially in the nocturnal period.

The system showed the ability to follow fast changes both in rabbits and in patients and wide oscillations of glucose levels in short times. The microfiber insertion was well tolerated by the human volunteer and no local infection was reported after its removal.

These preliminary in vivo experiments showed a satisfactory system performance and features: stability of the sensitivity, reproducibility, one point calibration, small walkman-like dimensions, the weight of only 245 g and the easiness of use. All these features allow us to enter into a multicenter European trial for clinical studies.

## References

Claremont, D.J., Sambrook, I.E., Penton, C., Pickup, J.C., 1986. Subcutaneous implantation of a ferrocene-mediated glucose sensor in pigs. Diabetologia 29, 817–821.

Clarke, W.L., Cox, D., Gonder-Fredrick, L.A., Carter, W., Pohl, S.L., 1987. Evaluating clinical accuracy of systems for self-monitoring of blood glucose. Diabetes Care 10 (5), 622.

Fogt, E.J., Dodd, L.M., Jenning, E.M., Clemens, A.H., 1978. Development and evaluation of glucose analyzer for a glucose controlled insulin infusion system (biostat). Clin. Chem. 24, 1366–1371.

Garg, S.K., Potts, R.O., Ackerman, N.R., 2002a. Correlation of fingerstick blood glucose measurements with GlucoWatch biographer glucose results in young subjects with type 1 diabetes. Diabetes Care 22, 11708–11714.

Garg, S.K., Fischer, J.S., Ruchti, T.L., Blank, T.B., Lorenz, A.D., Monfre, S.L., 2002. Calibration Break-Through for Near-Infrared, Non-Invasive Blood Glucose Monitoring. Diabetes Abstract Book 62nd Scientific Sessions 14–18 June 2002.

Johnson, K.W., Mastrototaro, J.J., Howey, D.C., Brunelle, R.L., Berden-Brady, P.L., Bryan, N.A., Andrew, C.C., Rowe, H.M., Allen, D.J., Noffke, B.W., McMaham, W.C., Morff, R.J., Lipson,

D., Nevin, R.S., 1993. In vivo evaluation of an electroenzymatic glucose sensor implanted in subcutaneous tissue. Biosens. Bioelectron. 7, 709–714.

Koudelka, M., Rohner-Jeanrenaud, E., Terrettaz, J., Bobbioni-Harsch, E., De Rooij, N.F., Jeanrenaud, B., 1991. In vivo behaviour of hypodermically implanted microfabricated glucose sensors. Biosens. Bioelectron. 6, 31.

Meyer, C., Bischof, F., Mennel, F.J., Sternberg, F., Pfeiffer, E.F., 1993. Use of microdialysis technique in the monitoring of subcutaneous tissue glucose concentration. Int. J. Artif. Organs 16, 268–275.

Pfeiffer, E.F., Meyerhoff, C., Bischof, F., Keck, F.S., Kerner, W., 1993. On line continuous monitoring of subcutaneous tissue glucose is feasible by combining portable glucosensor with microdialysis. Horm. Metab. Res. 25, 121–124.

Pickup, J.C., 1993. Developing glucose sensor for in vivo use. Trends Biotechnol. 11, 285–291.

Pickup, J.C., Alcock, S.J., 1991. Clinicians requirements for in vivo monitoring. Biosens. Bioelectron. 6, 639–646.

Pickup, J.C., Shaw, C.W., Clameront, D.J., 1989. In vivo molecular sensing in diabetes: an impiantable glucose sensor with direct electron transfer. Diabetologica 32, 213–217.

Shaw, C.W., Clameront, D.J., Pickup, J.C., 1991. In vitro testing of a simply constructed highly stable glucose sensor suitable for implantation in diabetic patients. Biosensors 6, 401–406.

The DCCT Research Group, 1993. The effect of intensive treatment on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New Engl. J. Med. 329, 977.

Turner, A.P.F., Pickup, J.C., 1980. Diabetes mellitus: biosensors for research and management. Biosensors 1, 85.