Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1377–1383, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00
© 1990 Pergamon Press plc

Extracorporeal determination of glucose, lactate and potassium with electrochemical biosensors*

MARCO MASCINI,†‖ GIUSEPPE PALLESCHI,‡ DANILA MOSCONE‡ and LUIGI BERNARDI§

† *Istituto di Chimica Analitica, Università di Firenze, Via G. Capponi 9, 50121 Firenze, Italy*
‡ *Dip. di Scienze e Tecnologie Chimiche, II Università di Roma, Via O. Raimondo, 00173 Roma, Italy*
§ *Istituto di Patologia Medica, Università Cattolica, Roma, Italy*

Abstract: Glucose, lactate and potassium ions have been continuously measured in whole blood by using two extracorporeal electrochemical biosensors and a new flow through potassium electrode. For experiments involving the "glucose clamp", lactate and potassium electrodes have been connected in series to an endocrine artificial pancreas "Betalike" properly modified. The same artificial pancreas has been used in experiments measuring the anaerobic threshold (AT) by continuous monitoring of lactate in athletes. In this case glucose and lactate sensors were connected in series for continuous measurement of both metabolites in blood in real time. The results are in agreement with the biochemical pathways involving such metabolites.

Keywords: *Glucose sensor; lactate sensor; potassium electrode;* in vivo *analysis; artificial pancreas; biosensors; sport medicine; glucose clamp.*

Introduction

A newly developed artificial pancreas, the Betalike (ESAOTE Biomedica, Ansaldo Div., Genova, Italy), has already been used in monitoring metabolites in whole blood using appropriate biosensors in extracorporeal experiments [1]. The device was designed for hospital use. It is fully computerized and simulates the beta cell function by continuously measuring the glycemic values of the patient, infusing the amount of insulin and/or glucose necessary to reach and maintain the selected glycemic values. The Betalike does not involve any blood loss from the patient because a miniaturized hemofiltering cartridge (Fig. 1) operates with blood drawn from one peripheral vein and returned to another. The instrument has been modified and used in automatic mode in the "glucose clamp" (GC) and in the determination of the aerobic and the anaerobic threshold (AT). The GC maintains the blood glucose concentration of the patient constant by infusion of a prefixed amount of insulin at constant rate. At the beginning of the experiment the glucose concentration of the patient is raised (130 mg dl^{-1}) and kept

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
‖To whom correspondence should be addressed.

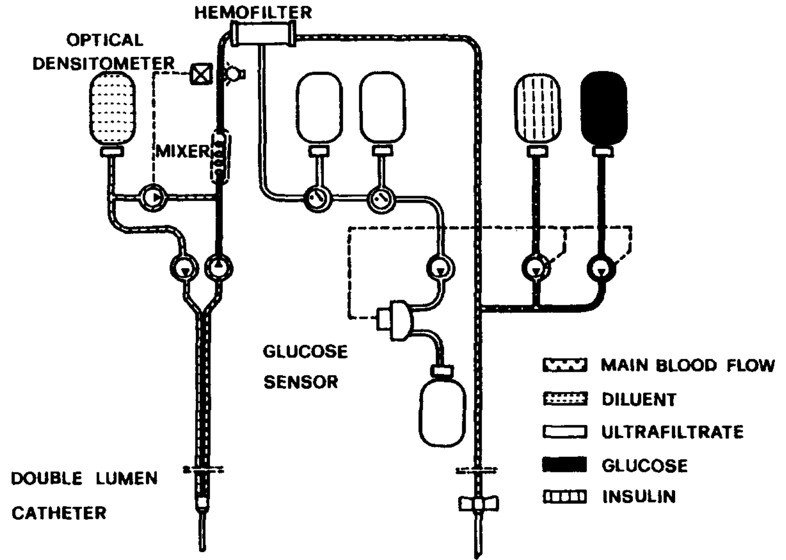

Figure 1
Principle of operation. The "Betalike" operates similarly to a hemodialysis system in drawing a small amount of blood from a peripheral vein and returning it via another vein. In order to prevent coagulation in the tubes the blood is diluted and heparinized at the tip of the drawing needle. The glucose measurements are carried out on the ultrafiltrate liquid obtained from a micro hemofilter cartridge. On a minute basis the Betalike infuses into the blood stream the amounts of insulin and glucose, calculated by means of a mathematical algorithm, which are needed to reach and maintain the selected glucose level.

constant during the insulin infusion by a glucose feed back pump regulated by an IBM computer connected to the Betalike. With this system it is possible to control the sensitivity of the patient to the insulin infusion.

Another experiment run with the "Betalike" was the determination of the AT in volunteers involved in sport competitions. Lactic acid was demonstrated to be the product of the activation of the anaerobic glycolysis pathway and it has been shown that its concentration increases dramatically when the exercise intensity reaches a fixed threshold [2–4]. The AT is defined as the highest VO (maximal oxygen uptake) beyond which increasing the lactate concentration in blood causes a metabolic acidosis [5]. This paper describes the continuous monitoring of lactic acid by substituting in the Betalike the glucose probe with a lactate probe. In this case the insulin and glucose feed-back pumps were disconnected and the computer was adapted for monitoring the lactate concentration. The Betalike was then used as an instrument withdrawing blood from the volunteer by a double lumen catheter diluting 10-fold, ultrafiltering and analysing the lactate content. A glucose electrode was added in series for monitoring both lactate and glucose from 20–30 min in athletes simulating races.

Experimental

Assembly of the lactate sensor

The surface of the platinum wire was covered with a membrane of cellulose acetate to eliminate interference from electroactive chemicals such as ascorbic acid, uric acid and glutathione. A second nylon membrane with immobilized lactate oxidase for the lactate

probe was placed on top of the first (the procedure of immobilizing enzymes on a nylon membrane has been described elsewhere [6]. The third membrane (outer) acted as a dialyser preventing microbial degradation of the immobilized enzyme. The three layers were held together with a rubber O-ring.

Assembly of the potassium sensor

A silicon tube of 1.5 mm inner diameter and 0.1 mm in thickness was filled with a 2.5% w/v solution of valinomycin in chloroform. The chloroform provoked a swelling of the silicon tube allowing the valinomycin to be absorbed into the silicon. In 30 min the chloroform evaporated and the silicon tube returned to its original form entrapping the valinomycin in its structure. The tube was then inserted into a polyethylene T tube and the extremities sealed with a silicon glue (Fig. 2). The T tube was filled with an aqueous solution of potassium chloride (10 mmol l^{-1}) and closed on the top with a silver/silver chloride inner reference electrode.

The outer SCE reference electrode, suitable for flow through systems, was placed downstream immediately before the waste bottle, in order to avoid potassium interference.

For the GC experiments the lactate and potassium electrodes were placed in series with the Betalike glucose probe. Calibration curves for both the electrodes were run before and after the experiment. An IBM computer connected with the artificial pancreas processed the data for the glucose infusion. For the AT experiments a glucose electrode was connected in series to the lactate probe placed in the Betalike.

Results and Discussion

Glucose clamp

The analytical behaviour of the potassium electrode during the GC experiment was excellent. This flowthrough silicon electrode showed a Nernstian slope from 0.01 to 10 mmol l^{-1} (Fig. 3) and no drift during the experiments. It has been hypothesized that the PVC liquid potassium membrane electrodes in the blood stream would drift due to

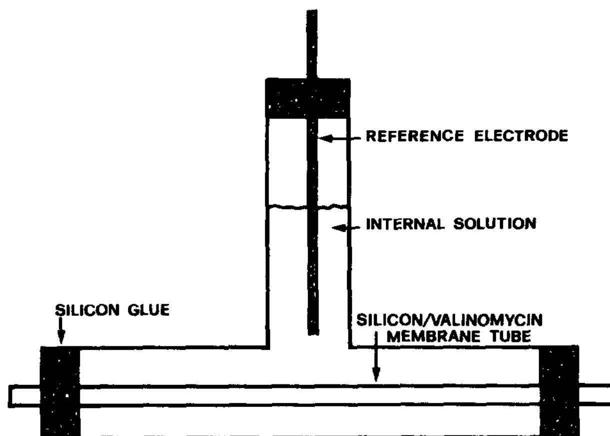

Figure 2
Schematic diagram of the flowthrough potassium electrode.

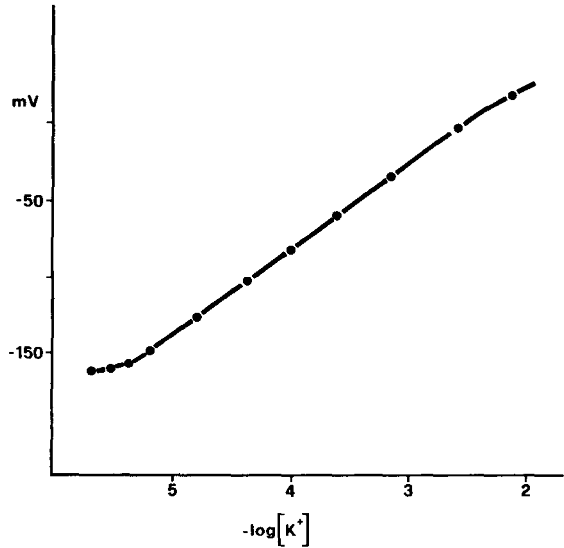

Figure 3
Flowthrough potassium electrode calibration curve.

the continuous extraction of softner and/or the carrier, with consequent lack of reproducibility. Moreover the life of the electrode might be drastically reduced.

The electrode prepared by using a silicon tube and the swelling technique showed no decrease in sensitivity after a 3 h experiment (see Fig. 4, calibrations before and after the GC experiment).

It is interesting to note that during the glucose load, at the beginning of the experiment (priming), in some cases when the glucose concentration was suddenly increased from 80 to 130 mg dl^{-1}, the potassium concentration decreased. The potassium decrease is in agreement with the theory that when there is an insulin infusion the potassium moves into the cells together with glucose with a consequent decrease of its concentration in blood. It is evident that the continuous monitoring of potassium in a patient with a low concentration of this metabolite, could be helpful (i.e. in diabetes) to avoid complications due to the hypokalaemia. In these experiments during the increase of glucose, an increase of lactate has been observed.

Anaerobic threshold

Lactate was monitored (Fig. 4) in the blood to an amateur athlete by asking him to run on a tread-mill at an initial speed of 8 km h^{-1}. He was then asked to increase his velocity by 1 km h^{-1} every 5 min. After 20 min the lactate concentration increased and in 5 min reached 3.6 mmol l^{-1} (4 mmol l^{-1} lactate concentration is conventionally considered by some authors as the starting point for the AT). After running ceased the lactate level returned to "normal" (Fig. 5).

In an analogous experiment a marathon runner varied his speed several times (Fig. 6) and at a running speed of 18 km h^{-1}, the lactate concentration rose dramatically. The glucose concentration remained constant. After the performance, the lactate concentration decreased rapidly while the glucose concentration increased. The behaviour of lactate and glucose during similar experiments has been described in the literature [7, 8]

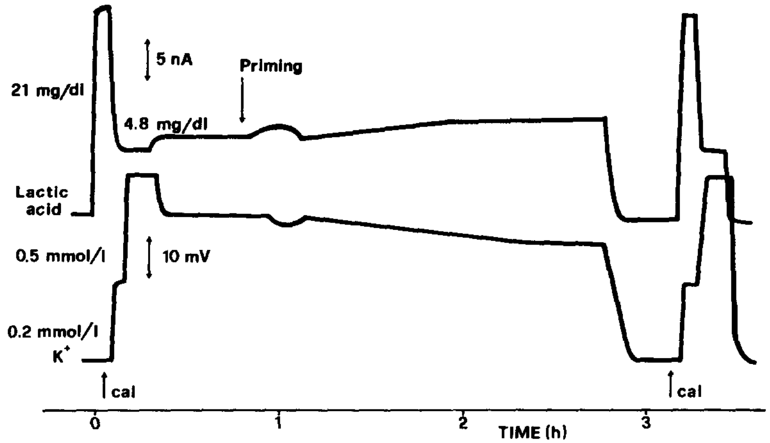

Figure 4
Continuous monitoring of lactic acid and potassium ions *in vivo* during a glucose-clamp experiment with the artificial pancreas "Betalike" and a flowthrough potassium electrode. See text for explanations.

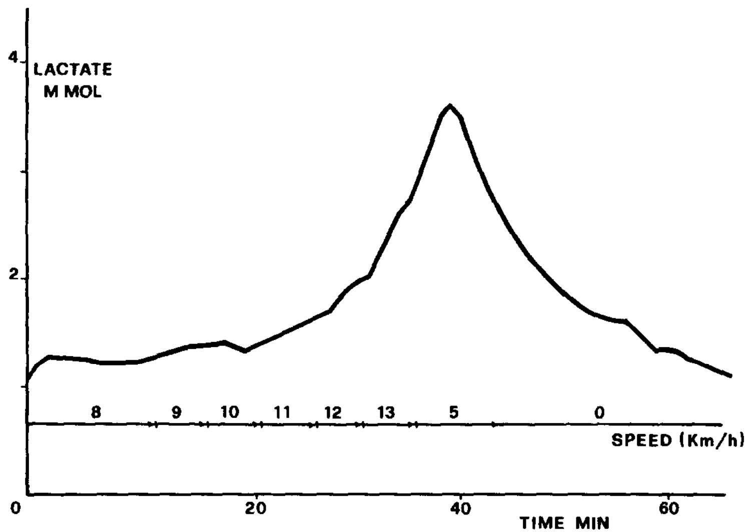

Figure 5
Continuous monitoring of lactic acid in an amateur athlete during a preliminary experiment for the measurement of the anaerobic threshold with the artificial pancreas.

but here it has been observed *in vivo*, continuously for the first time, using two biosensors.

Thirteen subjects aged from 24 to 56 years were studied on the tread-mill. Nine were athletes of high level (three marathon, two triathlon and four 10,000 m track athletes). Two of the subjects were physically active, and two had sedentary occupations.

The experimental method produces a precise real time picture of lacticacidemia during athletic physical exercise. Moreover it is possible to identify the onset of blood lactate accumulation (OBLA) fixed by some authors to a lactate concentration of 2 mmol l^{-1}

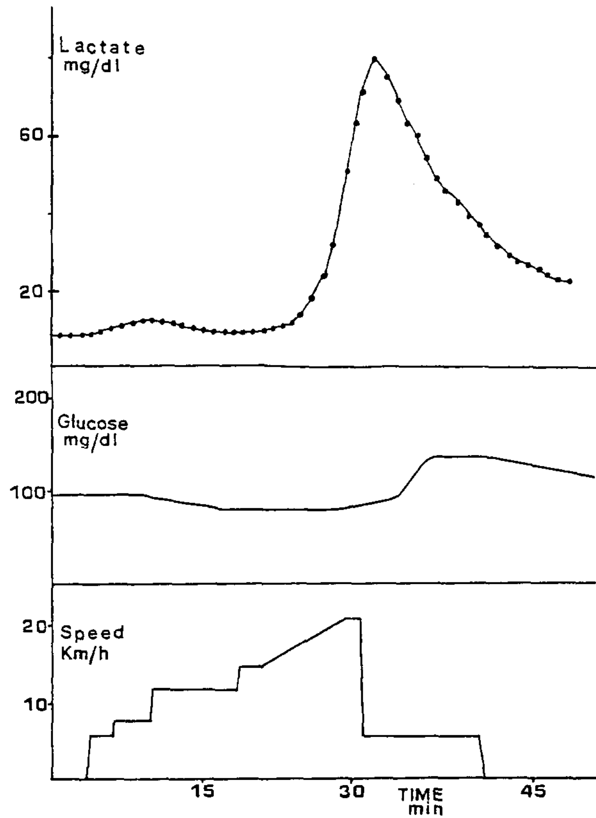

Figure 6
Continuous monitoring of lactic acid and glucose in a marathon runner during an "anaerobic threshold" experiment with the artificial pancreas Betalike. See text for explanations and procedure.

Table 1

n	Age	Sport	AT (km h^{-1})	LA (mg dl^{-1})
3	24–26	marathon	19.5	19.0
2	34–24	triathlon	17.0	16.9
4	25–40	10,000 m	15.2	16.0
2	24–26	active	11.0	15.0
2	26–41	sedentary	8.5	14.0

AT = Average speed (km h^{-1}) at "anaerobic threshold" determined to the point OBLA (onset blood lactate accumulation).
LA = Average of lactic acid concentration measured at the AT.

[9]. This point is identified as the aerobic threshold, while the point in which the lactate reaches a concentration of 4 mmol l^{-1} is conventionally defined as the starting point of the anaerobic threshold (AT) [3, 9–13]. The lactic acid variation in concentration from 2 to 4 mmol l^{-1} is well defined (Fig. 5) and an appreciable variation of the slope from 2 to 4 mmol l^{-1} lactate can be observed. This has also been observed for all the athletes in the experiment. Table 1 shows the average of AT and the concentration of lactic acid for the athletes tested.

As expected the marathon runners showed the highest speed at AT while the sedentary men displayed the lowest speed. This is in agreement with the fact that athletes well trained in long distance races maintain a relatively high running speed in aerobic conditions. It is also interesting to note that the lactate concentration calculated at the AT followed the same trends as the running speed showing that marathon runners can bear a high concentration of this metabolite during competition without reaching the AT.

These results, obtained by continuous monitoring of blood in real time using biosensors, open new possibilities for a better understanding of the metabolic glycolytic pathway, the altered concentration of several metabolites and their restoration to normal.

References

[1] M. Mascini, F. Mazzei, D. Moscone, G. Calabrese and M. M. Benedetti, *Clin. Chem.* **33**, 591 (1987).
[2] G. A. Brooks, *Med. Sci. Sports Exer.* **17**, 22 (1985).
[3] J. A. Davis and G. Gass, *Eur. J. Appl. Physiol.* **47**, 141 (1981).
[4] I. Jacobs, *Sports Med.* **3**, 10 (1986).
[5] J. A. Davis, M. H. Frank, B. J. Whipp and K. Wasserman, *J. Appl. Physiol.* **157**, 45 (1984).
[6] M. Mascini, M. Iannello and G. Palleschi, *Anal. Chim. Acta* **146**, 135 (1983).
[7] J. Wahren, *Diabetes* **28**, 82 (1979).
[8] N. Siliprandi, *Biochim. Clin.* **12**, 166 (1988).
[9] A. Mader, H. Liesen, H. Heck, A. Philippi, R. Rost, P. Schürch and W. Hollmann, *Labor Sportarzt Sportmed.* **27**, 80–112 (1976).
[10] J. A. Davis, *Med. Sci. Sports Exer.* **17**, 6–18 (1985).
[11] J. Karlsson and I. Jacobs, *Int. J. Sports Med.* **3**, 190–201 (1982).
[12] W. Kinderman, G. Simon and J. Keul, *Eur. J. Appl. Physiol.* **42**, 25–34 (1979).
[13] H. Stegman and W. Kinderman, *Int. J. Sport Med.* **3**, 105–110 (1982).

[Received for review 1 June 1989]