EUROPEAN ACHIEVEMENTS IN SENSOR RESEARCH DEDICATED TO IN VIVO MONITORING - (a) Glucose

J.C. Pickup, Daniel Thevenot

EUROPEAN ACHIEVEMENTS IN SENSOR RESEARCH DEDICATED TO *IN VIVO* MONITORING

(a) GLUCOSE

J.C. Pickup and D.R. Thévenot

OUTLINE

Advances in Biosensors
Supplement 1, pages 201–225

ISBN: 1-55938-630-4

1. METHODS

Members of the EC Concerted Action on Chemical Sensors for *In Vivo* Monitoring, and invited workshop participants, were sent a questionnaire which sought to record details of *in vivo* and *ex vivo* glucose sensors under development in Europe. One workshop participant working in the United States was also included.

The questionnaire was designed to elicit information about sensors for any analyte, but only the responses for glucose are presented here. Questions were asked about the clinical problem for which the sensor was being developed, the sensor operating principle and construction, the *in vitro* and *in vivo* operating characteristics, the retesting of the sensor after explantation and any *ex vivo* evaluation in flow-through cells.

2. SUMMARY OF RESULTS

2.1 Intended Use and General Operating Principles

Twenty-three designs of glucose sensor were described (Tables 1–5). Of these, about half had already been tested *in vivo* at the time of reporting, five designs had been evaluated *ex vivo* (which was their intended eventual use) and seven sensors had yet to reach to stage of either *in vivo* or *ex vivo* testing (and were therefore tested *in vitro* only). The vast majority of devices were being developed for use in patients with diabetes mellitus, though vital function monitoring in the intensive-care setting (which may include glucose and other metabolites, as well as oxygen, carbon dioxide and pH) and the research application of glucose sensors in neuroscience to monitor brain-glucose levels were also mentioned (Table 1).

Almost all sensors were amperometric enzyme electrodes, except for one enzyme thermistor (No. 12). The most popular base electrodes were made from platinum (74% of all devices) or carbon (30%), with a wire configuration usually employed for those sensors already tested *in vivo*. Chip-based sensors, which have perhaps greater potential for miniaturization and mass production, were, with one exception, confined to sensors at the *in vitro* stage of development. Table 2

shows that 8 of the 11 glucose sensors tested *in vivo* were hydrogen peroxide-detecting electrodes with catalysis by immobilized glucose oxidase and recording of current at an applied potential of +600–700 mV. Two *in vivo* devices were mediator-based, one using a ferrocene derivative and a set potential of +160 mV (No. 7) and the other using $TTF^{+}TCNQ^{-}$ at a potential of +250 mV (No. 1). Only one sensor (No. 3) was based on monitoring oxygen consumption by the glucose oxidase-catalysed oxidation of glucose. Here, the set potential was -600 mV. Ferrocene-mediated sensors were also being tested ex vivo and in vitro.

Inner membranes between the base electrode and the enzyme are commonly used for exclusion of co-reactants. It is interesting that 14 of the 23 sensors employed no such membrane (Table 2); but when used, cellulose acetate was the commonest inner membrane (4 devices). Outer membranes have multiple functions, including exerting a diffusion barrier to the analyte and thus extending linearity, preventing leakage of enzyme/mediator and determining the biocompatibility. Polyurethane and polycarbonate were amongst the polymer membranes often used for this purpose, but 9 sensors had no outer membrane.

Most *in vivo* sensors had Ag/AgCl as a reference electrode (Table 2) and when implanted this was either integrated with the working electrode (i.e. actually implanted) or applied to the skin surface of the animal or volunteer human subject (Table 4). One sensor employed a steel reference electrode (No. 11).

2.2 Operating Characteristics *In Vitro*

Table 3 shows that *in vivo* sensors generally had good linearity when calibrated *in vitro* (range 15–35 mM maximum glucose). Response times were variable but the upper limit was not unacceptable (2–300 s) and the reported loss of sensitivity when operated in buffer (drift) was low at 1%/h or less. Most sensors were insensitive to lowered oxygen tension to a value of 37.5 mm Hg (5 kPa) or less. These are characteristics which are generally regarded as desirable for application as an implantable glucose sensor for use in diabetes.

In vitro sensitivity varied nearly 700-fold for *in vivo* sensors

Table 1. Glucose sensors: their intended use and configuration.

No.	Principal Author	Country	Clinical Problem	Intended Use	Sensor Type	Base Electrode	Config-uration	Diameter (mm)
Glucose sensors used in vivo								
1	Fillenz	UK	neuroscience	*in vitro/vivo*	amp/enz	C	wire	0.3
2	Fischer	D	DM	*in vivo*	amp/enz	Pt	wire	2
3	Gough	US	DM, IC	*in vivo*	amp/enz	Pt	wire	1
4	Kerner	D	DM	*in vivo*	amp/enz	Pt	wire	0.4
5	Koudelka	CH	DM	*in vivo*	amp/enz	Pt	chip	0.9
6	Mascini	I	DM	*in vitro/vivo*	amp/enz	Pt	wire	0.5
7	Pickup	UK	DM	*in vivo*	amp/enz	Pt, C	wire	1.2
8	Pickup	UK	DM	*in vivo*	amp/enz	Pt	wire	0.5
9	Reach/Thévenot/Wilson	F	DM	*in vivo*	amp/enz	Pt	wire	0.25
10	Schmidt, F.J.	NL	DM	*in vivo*	amp/enz	Pt	flow cell	0.8
11	Vadgama	UK	DM/vital fn	*in vivo*	amp/enz	Pt	wire	0.5
	Mean							0.8
	SD							0.5
	No. sensors							11
	Minimum							0.3
	Maximum							2.0
Glucose sensors used ex vivo								
12	*Danielsson*	*SW*	*DM/decentr*	*in vitro/ex vivo*	ther/enz	therm	flow-cell	0.3
13	Fillenz	UK	neuroscience	*ex vivo*	amp/enz	C	flow cell	
14	Fischer	D	DM	*in vitro/ex vivo*	amp/enz	Pt	flow cell	8
15	Keck	D	DM	*ex vivo*	amp/enz	Pt	flow cell	0.4
16	Mascini	I	DM	*ex vivo*	amp/enz	Pt	flow-cell	3

Mean								2.9
SD								3.1
No. sensors								4
Minimum:								0.3
Maximum								8.0
Glucose sensors used in vitro								
17	*Jacobs*	*B*	DM/dial	*in vitro/vivo*	amp/enz	Pt	paste chip	
18	Kauffmann	B		*in vitro/ex vivo*	amp/enz	C	paste	3
19	Pfeiffer	D	DM	*in vitro/ex vivo*		Pt	wire	0.5
20	Schmidt, H-L	D		*in vitro/ex vivo*	amp/enz	C	wire	
21	Turner	UK	DM	*in vitro/vivo*	amp/enz	Pt-C	chip	3
22	Turner	UK	DM	*in vitro/vivo*	amp/enz	C, Au	chip	0.1
23	Urban	AU	DM, IC	*in vitro/vivo*	amp/enz	Pt	chip	
Mean								1,65
SD								1.36
No. sensors								4
Minimum:								0.10
Maximum								3.00

Key:
Country: UK, United Kingdom; D, Germany; US, United States; CH, Switzerland; I, Italy; F, France; NL, Netherlands; SW, Sweden; B, Belgium.
Clinical Problem: DM, Diabetes mellitus; IC, Intensive care; vital fn, vital function monitoring; decentr, decentralised testing; dial, dialysis.
Sensor type: amp/enz, amperometric enzyme electrode; ther/enz, enzyme thermister.
Working electrode: Pt, platinum; C, carbon; Au, gold; S, steel

Table 2. Construction of glucose sensors.

No.	Enzyme	Activity (U/mg)	Enzyme Immob.	Mediator	Membrane inner	Membrane outer	Application	Reference electrode	Electr. No.	Applied potential (mV)
In vivo										
1	GOx	300	adsorption	TTF-TCNQ	none	none	none	Ag-AgCl	3	250
2	GOx	130	GA Seph	none	none	PE-CA	D	Ag-AgCl	2	650
3	GOx/Cat		GA-albumin	none	Silastic	albumin	SH	Ag-AgCl	3	−600
4	GOx	250	GA	none	none	PU	D	Ag-AgCl	2	700
5	GOx	250	GA-BSA	none	none	PU	D	Ag-AgCl	3	700
6	GOx	137	GA	none	CA	PC	D + SH	Ag-AgCl	2	650
7	GOx	80	Seph	ferrocene	none	cellulose	SH	Ag-AgCl	2	160
8	GOx	80	entrap	none	none	PU-pHEMA	D	Ag-AgCl	2	700
9	GOx	250	GA	none	CA	PU	D	Ag-AgCl	2	650
10	GOx	250	none	none	none	none		Ag-AgCl	2	600
11	GOx	160	GA	none	PES	PU	D	S	2	650
Mean		189							2	465
SD		76							0	381
No.		10							11	11
Min		80							2	−600
Max		300							3	700
Ex vivo										
12	GOx/Cat	250	agarose CNBr	none	none	none	none	none	none	none
13	GOx	300	carbo	ferrocene	none	none		Ag-AgCl	3	0
14	GOx	130	GA-Seph	none	cellulose	cellulose	SH	Ag-AgCl	2	650
15	GOx	145	membrane	none	CA	PC	SH	Ag-AgCl	2	700
16	GOx	137	GA-nylon	none	CA	none	SH	Ag-AgCl	3	650

	Mean	192							2	400
	SD	69							1	327
	No.	5							5	5
	Min	130							0	0
	Max	300							3	700
	In vitro									
17	GOx		GA-BSA	none	none	none	D, SH, SP	Ag-AgCl	3	700
18	GOx	150	paste	ferrocene	none	PC		calomel	3	160
19	GOx	150	entrap PU	none	cellulose	PC, cellulose	SH	Ag-AgCl	2	600
20	GOx	245	covalent	none	none	none		calomel	3	600
21	GOx	125	GA	none	various	none		calomel		400
22	GOx	125	absorption	ferrocene	none	none		Ag-AgCl	3	420
23	GOx	223	chloranil	none	none	Nafion	D, SP	Ag-AgCl	3	300
	Mean	170							3	454
	SD	47							0	176
	No.	6							6	7
	Min	125							2	160
	Max	245							3	700

Key:
Enzyme: GOx, glucose oxidase; cat, catalase.
Enzyme immobilization method: GA, glutaraldehyde; Seph, Sepharose; BSA, bovine serum albumin; entrap, entrapment; carbo, carbodiimide; PU, polyurethane.
Membrane: CA, cellulose acetate; PES, polyether sulphone; PE, polyelthylene; PU, polyurethane; PC, polycarbonate; pHEMA, polyhydroxyethylmethacrylate.
Reference electrode: Ag/AgCl, silver/silver chloride; S, steel.
Membrane application: D, dip coating; SH, sheet; SP, spin coating.

Table 3. *In vitro* operating characteristics of glucose sensors.

No.	Glucose max (mM)	Response time (s)	Io (nA)	Sensitivity (nA/mM)	Relative sensitivity ($5.S/I_o$)	Lowest O_2 (mm Hg)	Drift (%/h)
Glucose sensors used in vivo							
1	35	2	1	25	125.0	—	0.2
2	20	180	1.2	1.3	5.4	15	1
3		300	2	5	12.5	—	0
4	16.5	24	0.7	1	7.1	25	10
5	18	30	1	2	10.0	37	—
6	20	60	0.05	0.1	10.0	—	1
7	20	266	25	11	2.2	94	1
8	30	35	30	70	11.7	37.5	0.5
9	15	210	1.3	2	7.7	8	0
10	30	60	100	4	0.2	—	0.1
11	30	120	0.3	0.15	2.5	30	0
Mean	23	117	15	11	18	35	1.4
SD	7	100	29	20	34	26	2.9
No.	10	11	11	11	11	7	10
Min	15	2	0.05	0.1	0.2	8	0
Max	35	300	100	70	125	94	10.0
Glucose sensors used ex vivo							
12	3	45	1 mV	100 mV/mM	500	—	0.1
13	10	2	0	200		—	0
14	2	20	0.5	1	10	—	1
15	16.7	420	0.5	1.2	12	40	0.2
16	20	30	1	8.5	42.5	—	1
Mean	10	103	0.4	42	141		0.5
SD	7	159	0.4	79	207		0.4
No.	5	5	5	5	4		5
Min	2	2	0.0	1	10		0
Max	20	420	1.0	200	500		1.0
Glucose sensors used in vitro							
17	12	60	50	1	0.1		—
18	100	20	300	50	0.8		0.5
19	24	10	1	5	25.0		5
20	10	10	5	20	20.0		0.07
21	8	12	100	650	32.5		
22	15	30		0.8			0.15
23	40	30	0.5	5	50.0		—
Mean	30	25	76	105	21		1.4
SD	30	17	106	223	17		2.1
No.	7	7	6	7	6		4
Min	8	10	1	1	0.1		0.1
Max	100	60	100	650	50		5.0

Key:
Glucose max, maximum glucose concentration at which response is linear; I_o, background current (current at zero glucose); relative sensitivity, response at 5 mM glucose/I_o; Lowest O_2, lowest p_{O_2} at which sensor response unaffected; drift, drift in buffered glucose solution.

(0.1–70 nA/mM), depending on such factors as working electrode type and area, but values ranging between 0.1 and 5 nA/mM were found with eight sensors used *in vivo*.

In order to gain more information about the responses of such sensors, we have calculated the ratio between their response to a 5 mM glucose increase, i.e. $5 \times S$, and the corresponding background current I_o (Table 3). This 5 x S/I_o ratio *in vitro*, ranged between 2.2 and 12.5 for the nine sensors tested *in vivo*; such values, although not high, are probably sufficient for glucose determinations under at least physiological conditions.

2.3 In Vivo Studies

Glucose sensors have been tested in a number of animal species (rat, dog, rabbit, sheep) but there was relatively little experience of studies in man (only four sensors) (Table 4). With the exception of the sensor used for neuroscience research (No.1), all devices had been sited in subcutaneous tissue, with two also used in a vein and one intraperitoneally. There was no general agreement about sterilization procedures, with glutaraldehyde, ethylene oxide, γ-irradiation, ethyl or isopropyl alcohol and no method, all being employed.

After a run-in time averaging 1.2 h, the implanted sensors were operated for periods of up to 108 d (Table 5). The latter sensor (No. 3) based on an O_2-consumption-detecting electrode used intravenously in the dog is exceptional. The nine glucose sensors implanted in the subcutaneous tissue of man or animals functioned from 0.2 to 10 d.

It is interesting to note in particular that the average sensitivity of 8 of 11 *in vivo* sensors was apparently reduced compared to initial calibration *in vitro* (20–93% of the *in vitro* value), thus necessitating *in vivo* calibration of sensors. In fact only three groups used a 2-point *in vivo* calibration procedure for their sensors, all others were satisfied with *in vitro* calibration or calibration in blood or plasma samples.

Sometimes, when sensors were recalibrated *in vitro* after explantation, the sensitivity was improved (three cases), but in most cases it was virtually the same as that *in vitro* prior to implementation.

This may indicate tissue factors which impair responses, rather than irreversible damage to the sensor caused by, for example, insertion

Table 4. Operating characteristics of *in vivo* and *ex vivo* glucose sensors.

No. recording	Species	Sterilisation method	Site	Anaesthetic	Reference Electrode Type	Run-in (h)	Method
In vivo *Characteristics*							
1	rat	none	brain	gen			non port
2	dog, rat	none	s.c	local	integral	4	tel, port
3	rat, dog, rabbit	GA	s.c., i.v.	gen, none	integral	0	tel, port
4	man, sheep	GA	s.c.	local	surface	2	non port
5	rat	gamma	s.c.	gen	integral	1	non port
6	rat	EtO	s.c.	gen	integral	0.5	non port
7	man	none	s.c.	local	surface	0.45	non port
8	man	none	s.c.	local	surface	1	portable
9	dog, rat	EtO, thiomersal	s.c./i.p.	gen	integ./surf.	2	portable
10	man	gamma, EtOH	s.c.	none	integral	0.5	tel, port
11	rat	gamma, IPA	s.c./i.v.	gen	integral	0.75	non port
Mean						1.2	
SD						1.1	
No. sensors						10	
Min						0.0	
Max						4.0	

Ex vivo *Characteristics*

12	man	none	i.v./capil.	none	integral	0.2	non port
13	rat	—	brain dialysate	none	integral	0	—
14	dog	none	i.v.	none	integral	0.5	non port
15	man, rat	GA	s.c. fluid	none		0.5	portable
16	man, rabbit	none	s.c. fluid	none		0	non port
Mean						0.2	
SD						0.2	
No. sensors						5	
Min						0.0	
Max						0.5	

Key:
GA, glutaraldehyde; EtO, ethylene oxide; EtOH, ethanol; IPA, isopropyl alcohol; s.c, subcutaneous; i.v., intravenous; i.p., intraperitoneal; capil, capillaries; port, portable recorder; tel, telemetry; gen, general anaesthetic; integral, reference and working electrode combined and both implanted; surface, reference electrode applied to skin surface.

Table5 . Operating characteristics of *in vivo* and *ex vivo* glucose sensors

No.	*In vivo* performance			Explanted sensor performance			Calibration	No. points
	Duration (d)	Sensitivity (nA/mM)	S *in vivo*/S *in vitro* (%)	*S/S in vitro* (%)	*T/T in vitro* (%)	*L/L in vitro* (%)		
In vivo *sensors*								
1	30	—	—	18	100	100	blood	—
2	10	0.8	62	95	110	100	IVV	dyn/2
3	108	5	100	100	100	100	IVT	—
4	0.3	0.2	20	85	65	85	plasma	—
5	7	1	50	95	100	95	IVV	1 or 2
6	0.2	0.05	50	50	200	100	IVT	2
7	0.25	6	55	203	106	—	IVT	1
8	0.3	65	93	60	—	—	IVT	1
9	10	0.5	25	100	100	100	IVV	2
10	—	2.5	63	100	100	—	blood	—
11	0.25	0.15	100	100	130	100	IVT	2
Mean	17	8	62	91	111	98		
SD	32	19	27	44	33	5		
No.	10	10	10	11	10	8		
Min	0.2	0.1	20	18	65	85		
Max	108	65	100	203	200	100		

ex vivo *sensors*

12	0.3	100[a]	100	100	100	100	IVT	1
13	30	1800	900	100	100	100	—	—
14	150	1.0	100	100	100	100	EVV	2
15	3	1.2	100	95	105	100	IVT	1
16	1	8.5	100	100	100	100	EVV	2
Mean	37	453	260	99	101	100		
SD	58	778	320	2	2	0		
No	5	4	5	5	5	5		
Min	0.3	1	100	95	100	100		
Max	150	1800	900	100	105	100		

Key:
Sin vivo/S*in vitro*=*in vivo* sensitivity as percentage of *in vitro* sensitivity; S/S*in vitro*=*in vitro* sensitivity after explantation/*in vitro* sensitivity prior to implantation; T/T*in vitro*=*in vitro* response time after explantation/*in vitro* response time prior to implantation; L/L*in vitro*=*in vitro* linearity after explantation/*in vitro* response time prior to implantation; IVV=*in vivo;* IVT=*in vitro;* EVV=*ex vivo;* dyn=dynamic regression analysis
[a]mV/mM.

procedures. Some devices had unchanged or worsened sensitivity after explantation, and here damage to the sensor during insertion, operation or removal cannot be excluded.

2.4 Ex Vivo Studies

Only five sensors were reported to have been tested extensively *ex vivo* in a flow-cell configuration (Tables 4 and 5). Three of these had been evaluated in man. The fluid sensed was blood, or brain or subcutaneous interstitial tissue fluid dialysate. The run-in time appeared to be shorter for these *ex vivo* devices (0–0.5 h) compared to the above implanted sensors. One sensor had been operated for up to 150 d (No. 14).

In contrast to implanted glucose sensors, the sensitivity of sensors operated *ex vivo* was generally the same as that *in vitro*.

3. CONCLUSIONS

This questionnaire demonstrates that a comparatively large number of implantable glucose sensors are under development in Europe. Although amperometric enzyme electrodes are the main focus of research, several different configurations such as chip, wire, paste and flow-through cell, are being investigated. The *in vitro* performance of these sensors is generally excellent, with good stability and linearity. However, less than half of the devices have been tested as *in vivo* sensors and, of these, only four have been evaluated in man.

Although first trials of implanted glucose sensors are very encouraging, a number of difficulties have emerged, such as a usually lowered sensitivity *in vivo* and hence the need for *in vivo* calibration procedures. Sensors have generally not been operated for more than a few days *in vivo* and much needs to be learnt about short- and long-term biocompatibility and bioperformance.

REFERENCES

Fillenz et al. (Sensor Nos. 1, 13)

Boutelle, M.G., Stanford, C., Fillenz, M., Albery, W.J. and Bartlett, P.M. (1986) An amperometric enzyme electrode for monitoring brain glucose in the freely moving rat. Neurosci. Lett., 72, 283–288.

Boutelle, M.G., Zetterström,T. and Fillenz, M. (1989) Enzyme electrode for the assay of glucose in dialysate. J. Neurosci. Methods, 29, 304.

Boutelle, M.G., Vahabzadeh, A., Girdler, N. and Fillenz, M. (1990) Changes in rat hippocampus 5-HT and DOPAC during graded changes in brain glucose.

Boutelle M.G., Svensson, L. and Fillenz, M. (1990) In vivo neurochemical effects of tail pinch. J. Neurosci. Methods, 34, 151–157.

Albery, W.J., Boutelle, M.G., Durrant, S.L.T., Fillenz, M., Hopkins, A.R. and Mangold, B.P. (1990) Sensitive enzyme electrodes. Phil. Trans. R. Soc. Lond. A, 333, 49–61.

Fischer et al. (Sensor Nos. 2, 14)

von Woedtke, T., Fischer, U., Abe, P., et al. (1989) In situ calibration of implanted electrochemical glucose sensors. Biomed. Biochim. Acta, 48, 943.

Fischer, U. (1991) Fundamentals of glucose sensors. Diabetic Medicine, 8, 309–321.

Rebrin, K., Fischer, U., Hahn von Dorsche, H., von Woedtke, T., Abel, P. and Brunstein, E. (1992) Subcutaneous glucose monitoring by means of electrochemical sensors: fiction or reality? Biomedical Eng., 14, 33–40.

Rebrin, K., Fischer, U., von Woedtke, Th. and Abel, P. (1993) Glucose monitoring in dogs by means of subcutaneous electrodes: day-to-day variations in sensor function. Biosensors Bioelectron., in press.

von Woedtke, Th., Fischer, U., Brunstein, E., Rebrin, K. and Abel, P. (1993) Implantable glucose sensors; comparison between in vitro and in vivo kinetics. Int. J. Artific. Organs, in press.

Gough et al. (Sensor No. 3)

Ertefai, S. and Gough, D.A. (1989) Physiological preparation for studying the response of subcutaneously implanted glucose and oxygen sensors. J. Biomed. Eng., 11, 362–366.

Armour, J.C., Lucisano, J.Y., McKean, B.D. and Gough, D.A. (1990) Application of chronic intravascular blood glucose sensor in dogs. Diabetes, 39, 1519–1526.

Kerner et al. (Sensor No.4)

Kerner, W., Zier, H., Stienbach, G., et al. (1988) A potentially implantable enzyme electrode for amperometric measurements of glucose. Horm. Metab. Res., 20 (suppl.), 8.

Koudelka et al. (Sensor No.5)

Koudelka, M., Gernet, S. and de Rooij, N.F. (1989) Planar amperometric enzyme-based glucose microelectrode. Sensors Actuators 18, 157–165.

Koudelka, M., Rohner-Jeanrenaud, F., Terrettaz, J., Bobbioni-Harsch, E., de Rooij, M.F. and Jeanrenaud B. (1989) *In vivo* response of microfabricated glucose sensors to glycemia changes in normal rats. Biomed. Biochim. Acta, 48, 953–56.

Koudelka, M., Rohner-Jeanrenaud, F., Terrettaz, J., Bobbioni-Harsch, E., de Rooij, N.F. and Jeanrenaud, B. (1991) *In vivo* behaviour of hypodermically implanted microfabricated glucose sensors. Biosensors Bioelectron., 6, 31–36.

Mascini et al. (Sensor Nos. 6, 16)

Mascini, M. and Palleschi, G. (1989) Design and applications of continuous monitoring probes. Selective Electrode Review, 11, 191–264.

Palleschi, G., Mascini, M., Bernardi, L., Bombardieri, G. and De Luca, A.M. (1989) Glucose clamp experiments with electrochemical biosensors. Anal. Lett., 22 (6), 1209–20.

Palleschi, G., Pilloton, R., Mascini, M., Bernardi, L., Bombardieri, G., De Luca, A. and Zeppilli P. (1989) Biosensor applications in medicine by continuous monitoring of metabolites. In: Karube, I. (ed.), *Biosensors*, Vol. 14. Materials Research Society, pp. 3–13.

Mascini, M. and Selleri, S. (1989) Glucose biosensor with extended linearity. Anal. Lett., 22 (6), 1429–49.

Mascini, M., Palleschi, G., Moscone, D. and Bernardi, L. (1989) Extracorporeal determination of glucose, lactate and potassium with electrochemical biosensors. J. Pharm. Biomed. Anal., 7, 1337.

Mascini, M., Selleri, S. and Riviello, M. (1989) Glucose electrochemical probe with extended linearity for whole blood. J. Pharm. Biomed. Anal., 7, 1507–12.

Palleschi, G., Mascini, M., Bernardi, L. and Zeppilli, P. (1990) Lactate and glucose electrochemical biosensors for the determination of the aerobic and anaerobic threshold in runners. Med. Biol. Eng. Comput., 28, B25.

Mascini, M. and Moscone, D. (1990) Electrochemical biosensors: application to some real problems. In: Turner, A.P.F. (ed), *Advances in Biosensors*, Vol.1. JAI Press, London, pp. 00–00.

Palleschi, G., Moscone, D. and Mascini, M. (1991) Electrochemical biosensors for extra-corporeal measurements. Biochem. Trans., 19, 5–9.

Bernardi, L., Bombardieri, G., De Luca, A.M., Pilloton, R., Palleschi, G. and Mascini, M. (1993) Evaluation of a new system for the continuous monitoring of blood glucose, lactate and potassium. Int. J. Artif. Organ, in press.

Moscone, D., Pasini, M. and Mascini, M. (1993) Subcutaneous microdialysis probe coupled with glucose biosensor for *in vivo* continuous monitoring. Talanta, in press.

Pickup et al. (Sensor Nos. 7, 8)

Claremont, D.J., Shaw, G.W. and Pickup, J.C. (1988) Improving the stability of potentially-implantable glucose sensors. Diabetic Med., 5, 2.

Pickup, J.C. and Claremont, D.J. (1988) Progress towards *in vivo* glucose sensing with a ferrocene-mediated amperometric enzyme electrode. Hormone Metabol.Res. (Suppl. Series), 20, 34–36.

Pickup, J.C., Shaw, G.W. and Claremont, D.J. (1988) Implantable glucose sensors: choosing the appropriate sensing strategy. Biosensors, 3, 335–46.

Claremont, D.J. and Pickup, J.C. (1988) Ambulatory glucose monitoring: Progress, problems and solutions. Biomed. Meas. Inform. Control, 2, 201–06.

Pickup, J.C., Shaw, G.W. and Claremont, D.J. (1989) Potentially-implantable, amperometric glucose sensors with mediated electron transfer: Improving the operating stability. Biosensors, 4, 109–19.

Pickup, J.C., Shaw, G.W. and Claremont, D.J. (1989) *In vivo* molecular sensing in diabetes mellitus: An implantable glucose sensor with direct electron transfer. Diabetologia, 32, 213–17.

Pickup, J.C. (1989) Self-regulating insulin delivery. In: Prescott, L.F. and Nimmo, W.S. (ed.), *Novel Drug Delivery and Its Therapeutic Application*. Wiley, Chichester, pp. 314–22.

Pickup, J.C. (1989) Biosensors for diabetes mellitus. In: Wise, D.L. (ed.), *Applied Biosensors*. Butterworth, Boston, pp. 227–47.

Pickup, J. (1991). Glucose sensors. In: Pickup, J.C. and Williams, G., *Textbook of Diabetes*. Blackwell Scientific, Oxford, pp. 994–1005.

Shaw, G.W., Claremont, D.J. and Pickup, J.C. (1991) *In vitro* testing of a simply-constructed, highly-stable glucose sensor suitable for implantation in diabetic patients. Biosensors Bioelectron., 6, 401–06.

Pickup, J.C. (1991) Glucose sensors. In: Bradley, C., Christie, M. and Home, P.D. (eds.), *The Technology of Diabetes Care*. Harwood, Reading, pp. 27–37.

Pickup, J.C. and Alcock, S. (1991) Clinicians' requirements for chemical sensors for *in vivo* monitoring. Biosensors Bioelectron., 6, 639–46.

Pickup, J.C. (1991) Glucose sensors and closed-loop insulin delivery. In: Pickup, J.C. (ed.), *Biotechnology of Insulin Therapy*. Blackwell Scientific, Oxford, pp. 126–53.

Reach, Thévenot, Wilson et al. (Sensor No. 9)

Tallagrand, T., Sternberg, R., Reach, G. and Thévenot, D.R. (1988) Evaluation of implantable glucose enzyme-based sensors with extracorporeal blood shunt. Horm. Metab. Res. 20, 13–16.

Velho, G., Froguel, Ph., Thévenot, D.R. and Reach, G. (1988) *In vivo* calibration of a subcutaneous glucose sensor for determination of subcutaneous glucose kinetics. Diabetes Nutr. Metab. Clin. Exptl., 1, 227–33.

Sternberg, R., Bindra, D., Wilson, G.S. and Thévenot, D.R. (1988) Covalent enzyme coupling on cellulose acetate membranes for glucose sensor development. Anal. Chem., 60, 2781–86.

Velho, G., Froguel, Ph., Sternberg, R., Thévenot, D.R. and Reach G. (1989) *In vitro* and *in vivo* stability of electrode potentials in needle-type glucose sensors. Diabetes, 38, 164–71.

Sternberg, R., Barrau, M.-B., Gangiotti, L., Bindra, D.S., Wilson, G.S., Velho, G., Reach, G. and Thévenot, D.R. (1989) Study and development of multi-layer enzyme-based glucose needle-type microsensor. Biosensors 4, 27–40.

Velho, G., Froguel, Ph., Thévenot, D.R. and Reach, G. (1989) Strategies for calibrating a subcutaneous glucose sensor. Biomed. Biochim. Acta, 48(11–12) 957–64.

Velho, G., Froguel, Ph. and Reach, G. (1989) Determination of peritoneal glucose kinetics: implications for the peritoneal implantation of closed-loop insulin delivery systems. Diabetologia, 32, 331–36.

Wilson, G.S. and Thévenot, D.R. (1989) Unmediated amperometric enzyme electrodes. In: Cass, T. (ed.), *Biosensors: A Practical Approach*. IRL Press, Oxford, pp. 1–5.

Wilson, G.S., Reach, G. and Thévenot, D.R. (1991). Biosensor for intracorporal measure-ments: problems and strategies. Biochem. Soc. Trans. 19, 9–11.

Bindra, D.S., Zhang, Y., Wilson, G.S., Sternberg, R., Thévenot, D.R., Moatti, D. and Reach, G. (1991) Design and in vitro studies of a needle-type glucose sensor for subcutaneous monitoring. Anal. Chem., 63, 1692–96.

Poitout, V., Moatti, D., Velho, G., Reach, G., Sternberg, R., Thévenot, D.R., Bindra, D., Zhang, Y. and Wilson, G.S. (1991) *In vitro* evaluation of a glucose sensor implanted in the subcutaneous tissues of conscious dogs. Trans. Am. Soc. Artif. Organs, 37(3), M298–M300.

Moatti, D., Capron, F., Poitout, V., Reach, G., Bindra, D.S., Zhang, Y., Wilson, G.S. and Thévenot, D.R. (1992) Towards continuous glucose monitoring: in vivo evaluation of a miniaturized glucose sensor implanted for several days in rat subcutaneous tissue. Diabetologia, 35, 224–30.

Moatti, D., Velho, G., Reach, G. (1992) *In vitro* and *in vivo* demonstration of the interfering effect of ascorbate and acetaminophen on glucose detection by a sensor. Biosensors Bioelectron., 7, 345–52.

Schmidt, F.J. et al. (Sensor No. 10)

Schoonen, A.J.M., Schmidt, F.J., Hasper, H., Verbrugge, D.A., Tiessen, R.G., Lerk, C.F. (1990) Development of a potentially wearable glucose sensor for patients with diabetes mellitus: design and in vitro evaluation. Biosensors Bioelectron., 5, 37–46.

Aalders, A.L., Schmidt, F.J., Schoonen, A.J.M., Broek, I.R., Maessen, A.G.F.M., Doorenbos, H. (1991) Development of a wearable glucose sensor: studies in healthy volunteers and diabetic patients. Int. J. Artif. Organs, 14, 102–08.

Schmidt, F.J., Aalders, A.L., Schoonen, A.J.M., Doorenbos, H. (1992). Calibration of a wearable glucose sensor. Int. J. Artif. Organs. Submitted.

Vadgama et al (Sensor No. 11)

Churchouse, S., Mullen, W., Battersby, C. and Vadgama, P. (1986) Needle enzyme electrodes for biological studies. Biosensors, 2, 325-342.

Vadgama, P. (1988) Biosensors. In: Williams, D.L. and Marke, V. (eds.),*Principles of Clinical Biochemistry:Scientific Foundations*. Heinemann, Oxford, pp. 229–50.

Vadgama, P., Mullen, W.H., Churchouse, S.J. and Battersby, C. (1988) The glucose enzyme electrode: is simple peroxide detection at a needle sensor acceptable? Hormone Metabol. Res. (Suppl.), 20, 20–22.

Vadgama, P. (1988) Enzyme electrodes and their potential for medical exploitation. Measurement, 4, 154–59.

Vadgama, P. (1988) Diffusion limited enzyme electrodes. In: Guilbault, G.G. and Mascini, M. (eds.), *Analytical Uses of Immobilised Biological Compounds for Detection, Medical and Industrial Uses*. Reidel, Boston, pp. 359–77.

Vadgama, P. (1988) What does the biomedical area require of sensors? Anal.Proc., 25, 274–76.

Baker, M.K., Vadgama, P. (1988) Chemical sensors and their relevance to clinical measurement. J. Meas. Control, 21, 53–59.

Vadgama, P., Spoors, J., Tang, L.X. and Battersby, C. (1989) The needle glucose electrode: *in vitro* performance and optimisation for implantation. Biomed. Biochim. Acta, 48, 933–42.

McDonnell, M.B.and Vadgama, P. (1989) Membranes: separation principles and sensing. In: Thomas, J.D.R. (ed.), *Selective Electrode Review*, Vol. 11. Pergamon Press, Oxford, pp. 17–67.

Vadgama, P. (1989) Biosensors—artificial sniffer dogs! Biol. Sci. Rev., 1 (5), 36–39.

Tang, L.X.and Vadgama, P. (1990) Use of membrane techniques for optimisation of enzyme electrodes. In: Wise, D.L. (ed.), *Bioinstrumentation: Research, Developments and Applications*. Publisher, Place, pp. 211–232.,

Vadgama, P. (1990) Membrane based sensors: a review. J.Membr. Sci., 50, 141–52.

Rolfe, P. and Vadgama, P. (1990) Editorial: Biosensors. Med. Biol. Eng. Comput., 28, B1–B2.

Vadgama, P. (1990) Biosensors: Adaptation for practical use. Sensors Actuators, B1, 1–7.

Vadgama, P., Desai, M., Koochaki, Z. and Treloar, P. (1991) Problems of data interpretation. Biochem. Soc. Trans., 19 (1), 11–15.

Vadgama, P., Desai, M.A., Christie, I. and Koochaki, Z. (1991) Chemical sensors and biosensors: nearer the patients. Pure Appl. Chem., 63 (8), 1147–52.

Mutlu, M., Mutlu, S., Rosenberg, M.F., Kane, J., Jones, M.N. and Vadgama, P. (1991) Matrix surface modification by plasma polymerisation for enzyme immobilization. J. Mater. Chem., 1 (3), 447–50.

Vadgama, P. (1992) Blood gas analysis. In: *Concise Encyclopaedia on Biological and Biomedical Measurement Systems*. In press.

Vadgama, P. and Desai, M. (1992) In vivo biosensors. In: Blum, L.J. and Coulet, P.R. (eds.), *Handbook of Biosensors*.

Vadgama, P., Desai, M. and Crump, P. (1992) Electroanalytical transducers for in vivo monitoring. In: Electroanalysis. In press.

Koochaki, Z. and Vadgama, P. (1992) The diffusion limited oxidase-based glucose enzyme electrode: relation between covering membrane permeability and substrate response. J. Membr. Sci., in press.

Danielsson et al. (Sensor No. 12)

Danielsson, B. (1990) Calorimetric biosensors. J. Biotechnol., 15, 187–200.

Keck et al. (Sensor No. 15)

Keck, F.S., Kerner, W., Meyerhoff, C., Zier, H. and Pfeiffer, E.F. (1992) A device for continous registration of dissolved glucose. Horm. Metab. Res., in press.

Jacobs et al. (Sensor No.17)

Sansen, W., Claes, A., De Wachter, D., Callewaert, L. and Lambrechts, M. (1989) A Smart sensor for biomedical applications. Proc. 11th Ann. Int. Conf. IEEE-EMBS, Seattle, WA, Nov. 1989.

Sansen, W., Lambrechts, M., Claes, A., De Wachter, D., and Callewaert, L. (1990). A smart sensor for the voltammetric measurement of oxygen or glucose concentrations. Transducers '90, 5th Int. Conf. on Solid-State Sensors and Actuators.

Kauffman et al. (Sensor No. 18)

Amine, A., Kauffman, J.-M. and Patriarche, G.J. (1991) Amperometric biosensor for glucose based on carbon paste modified electrodes. Talanta, 38, 107–110.

Pfeiffer et al. (Sensor No. 19)

Scheller, F., Schubert, F., Pfeiffer, D. et al. (1989) Research and development of biosensors: a review. Analyst, 114, 653.

Pfeiffer, D., Setz, K., Klines, N. et al. (1992) Enzyme electrodes for medical applications. *GBF Monographs 14*, in press.

Schmidt, H.-L. et al. (Sensor No. 20)

Schuhmann, W., Wohlschl ger, H., Lammert, R., Schmidt, H.-L., Löffler, U., Wiemmhöfer, H.-D. and Göpel, W. (1990) Leaching of dimethylferrocene, a redox mediator in amperometric enzyme electrodes. Sensors Actuators, B1, 571–75.

Schuhmann, W., Lammert, R., Uhe, B. and Schmidt, H.-L. (1990) Polypyrrole, a new possibility for covalent binding of oxidoreductases to electrode surfaces as a base for stable biosensors. Sensors Actuators, B1, 537–41.

Schuhmann, W. (1991) Functionalized polypyrrole. A new material for the construction of biosensors. Synthetic Metals, 41, 429–32.

Schuhmann, W. and Kittsteiner-Eberle R. (1991) Evaluation of polypyrrole/glucose oxidase electrodes in flow-injection systems for sucrose determination. Biosensors Bioelectron., 6, 263–73.

Schuhmann, W., Ohara, T.J., Schmidt, H.-L., Heller, A. (1991). Electron transfer between glucose oxidase and electrodes via redox mediators bound with flexible chains to the enzyme surface. J. Amer. Chem. Soc., 113, 1394–97.

Schuhmann, W. (1991) Amperometric substrate determination in flow-injection systems with polypyrrole-enzyme electrodes. Sensors Actuators, B4, 41–49.

Schuhmann, W., Lammert, R., H mmerle, M. and Schmidt, H.-L. (1992) Electrocatalytic properties of polypyrrole in amperometric electrodes. Bioelectronics, 6, in press.

Turner et al. (Sensor Nos. 21, 22)

Turner, A.P.F. (1987) Amperometric biosensors on modified porous graphite electrodes. Ann. N.Y. Acad. Sci., 501, 551–52.

Turner, A.P.F., D'Costa, E.J and Higgins, I.J. (1987). Enzymatic analysis using quinoprotein dehydrogenase. Ann. N. Y. Acad. Sci., 501, 283–87.

Turner, A.P.F., Hendry, S.P. and Cardosi, M.F. (1987) Tetrathiafulvalene: a new mediator for amperometric biosensors. In: *Biosensors, Instrumentation and Processing: The World Biotech Report*, Vol. 1, Number 3. Online, London, pp. 125–37.

Hendry, S.P., and Turner, A.P.F. (1988) A glucose sensor utilising tetracyanoquinodimethane as a mediator. Hormone Metabol.Res., 20, 37–40

Turner, A.P.F. (1988) Amperometric biosensors based on mediator modified electrodes. *Methods in Enzymology*, Vol. 137. Academic Press, London, pp. 90–103.

Dicks, J.M., Hattori, S., Karube, I., Turner, A.P.F. and Yokozawa, T. (1989) Ferrocene modified polypyrrole with immobilised glucose oxidase and its application in amperometric glucose microbiosensors. Ann. Biol. Clin., 47, 607–19.

Palleschi, G. and Turner, A.P.F. (1990) Amperometric tetrathiafulvalene-mediated lactate electrode using lactate oxidase absorbed on carbon foil. Analyt. Chim. Acta, 234, 459–63.

Hu, J. and Turner, A.P.F. (1991) An enzyme electrode for glucose consisting of glucose oxidase immobilised at a benzoquinone-modified carbon electrode. Analyt. Lett., 24, 15–24.

Hendry, S.P., Cardosi, M.F., Neuse, E.W and Turner, A.P.F. (1992) Polyferrocenes as mediators in amperometric biosensors for glucose. Analyt. Chim. Acta (in press).

Morris, N.A., Cardosi, M.F., Birch, B.J. and Turner, A.P.F. (1992). Electrochemical capillary fill device for the analysis of glucose incorporating glucose oxidase and ruthenium (III) hexamine as mediator. Electroanalysis, 4, 1ed>9..

Newman, J.D., Marraza, G. and Turner, A.P.F. (1992) Ink-jet printing for the fabrication of amperometric glucose biosensors. Analyt. Chim. Acta, 262, 13–17.

Turner, A.P.F., Karube, I. and Wilson, G.S. (eds>) (1989). *Biosensors: Fundamentals and Applications*. Oxford University Press, Oxford. p. 770.

Alcock, S.J. and Turner, A.P.F. (1992). *Proceedings of the European Concerted Action on Chemical Sensors for in vivo Monitoring*. Cranfield Press, Cranfield.

Turner, A.P.F. (1992). Biosensor research in the UK. In: Mascini, M. (ed.), *Biosensor Research in Europe*. CEC, Brussels.

Newman, J.D. and Turner, A.P.F. (1992). *Recent Advances in UK Chemical and Biosensor Technology*. ASTTP Study, DTI, London.

Cardosi, M.F. and Turner, A.P.F. (1987). Glucose sensors for the management of diabetes. In: Alberti, K.G.M.M. and Krall, L.P.(eds.), *The Diabetes Annual*, Vol. 3. Elsevier, Amsterdam, pp. 560–78.

Turner, A.P.F. (1987) Miniaturised sensors. In: Brunetti, P. and Waldhausl, W. (eds.), *Advanced Models for the Therapy of Insulin-dependent Diabetes*. Raven Press, New York, pp. 229–34.

Coughlan, M.P., Kierstan, M.P.J., Border, P.M. and Turner, A.P.F. (1988) Analytical applications of immobilised proteins and cells. J. Microbiol. Meth., 8, 1–50.

Turner, A.P.F. (1989) Current trends in biosensor research and development. Sensors Actuators, 17, 433–50.

Cardosi, M.F. and Turner, A.P.F. (1990). Recent advances in enzyme-based electrochemical glucose sensors. In: Alberti, K.G.M.M and Krall, L.P. (eds.), *The Diabetes Annual*, Vol. 5. Elsevier, Amsterdam, pp. 254–72.

Cardosi, M.F. and Turner, A.P.F. (1991) The development of implantable amperometric glucose sensors. In: Alberti, K.G.M.M. and Krall, L.P. (eds.), *The Diabetes Annual*, Vol. 6. Elsevier, Amsterdam. pp. 271–301.

Cardosi, M.F. and Turner, A. P. F. (1991). Mediated electrochemistry: A practical approach to biosensing. In: Turner, A.P.F. (ed.), *Advances in Biosensors*, Vol. 1. JAI Press, London, pp. 125–69.

Alcock, S.J., Danielsson, B. and Turner, A.P.F. (1992). Advances in the use of *in vivo* sensors: Review of recent *in vivo* and *ex vivo* results, ethical, safety and technical problems. Biosensors Bioelectron., 7, 243–54.

Wilson, R. and Turner, A.P.F. (1992). Glucose oxidase: An ideal enzyme. Biosensors Bioelectron., 7 (2).

Jaffari, S.A. and Turner, A.P.F. (1992) Printed glucose sensor incorporating platinised carbon and glucose oxidase. In: *Biosensors '92*. Elsevier, Oxford.

Urban et al. (Sensor No. 23)

Buxbaum, E., Jachimoxicz, A., Jobst, G., Olcaytug, F., Pittner, F., Schalkhammer, T. and Urban, G. (1990) New microminiaturized glucose sensors using covalent immobilization techniques. Sensors Actuators, B1, 518–22.

Pifl, C., Jachimoxicz, A., Urban, G., Kohl, F., Goiser, P., Theiner, J. and Nauer, G. (1990) A new type of a thin film microelectrode for in vivo voltammetry. Sensors Actuators, B1, 468–72.

Schalkhammer, T., Mann-Buxbaum, E., Urban, G. and Pittner, F. (1990). Electrochemical biosensors on thin film metals and conducting polymers. J. Chromatogr., 510, 355–66.

Schalkhammer, T., Mann-Buxbaum, E., Urban, G. and Pittner, F. (1990) Biosensors on thin film metals and polymer coated electrodes. Fresenius J. Anal. Chem., 337, 107.

Urban, G., Klepinger, F., Kohl, F., Kuttner, H., Jobst, G., Pittner, F., Schalkhammer, T. and Mann-Buxbaum, E. (1990) Miniaturised sensors to record metabolic variables. J. Clin. Monitor., 6 (2), 163–64.

Urban, G., Kamper, H., Jachimoxicz, A., Kohl, F., Kuttner, H., Olcaytug, F., Goiser, P,, Pittner, F., Schalkhammer, T. and Mann-Buxbaum, E. (1991) The construction of microcalorimetric biosensors by use of high resolution thin-film thermistors. Biosensors Bioelectron., 6, 275–80.

Schalkhammer, T., Mann-Buxbaum, E., Pittner, F., Urban, G. (1991). Electrochemical glucose sensors on permselective non-conducting substituted pyrrole polymers. Sensors Actuators, B4, 273–81.

Urban, G., Jobst, G., Kohl, F., Jachimoxicz, A., Olcaytug, F., Tilado, O., Goiser, P., Nauer, G., Pittner, F., Schalkhammer, T. and Mann-Buxbaum, E. (1992) Miniaturised thin-film biosensors using covalently immobilized glucose-oxidase. Biosensors Bioelectron., in press.