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

(a) GLUCOSE

J.C. Pickup and D.R. Thévenot

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## OUTLINE

<b>1. Methods</b>	<b>202</b>
<b>2. Summary of Results</b>	<b>202</b>
2.1 Intended use and general operating principles	202
2.2 Operating characteristics <i>in vitro</i>	203
2.3 <i>In vivo</i> studies	209
2.4 <i>Ex vivo</i> studies	214
<b>3. Conclusions</b>	<b>214</b>
<b>References</b>	<b>215</b>

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

Mean  
SD  
No. sensors  
Minimum:  
Maximum

2.9  
3.1  
4  
0.3  
8.0

*Glucose sensors used in vitro*

17	Jacobs	B	DM/dial	in vitro/vivo	amp/enz	Pt	paste chip	3	1.65
18	Kauffmann	B		in vitro/ex vivo	amp/enz	C	paste	0.5	1.36
19	Pfeiffer	D	DM	in vitro/ex vivo		Pt	wire	3	4
20	Schmidt, H-L	D		in vitro/ex vivo	amp/enz	C	wire	0.1	0.10
21	Turner	UK	DM	in vitro/vivo	amp/enz	Pt-C	chip		
22	Turner	UK	DM	in vitro/vivo	amp/enz	C, Au	chip		
23	Urban	AU	DM, IC	in vitro/vivo	amp/enz	Pt	chip		3.00
	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 thermometer.  
 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		Application	Reference electrode	Electr. No.	Applied potential (mV)
					inner	outer				
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	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	D, SH, SP	Ag-AgCl	3	700
18	GOx	paste	ferrocene	none		calomel	3	160
19	GOx	entrap PU	none	cellulose	SH	Ag-AgCl	2	600
20	GOx	covalent	none	none		calomel	3	600
21	GOx	GA	none	various		calomel		400
22	GOx	absorption	ferrocene	none		Ag-AgCl	3	420
23	GOx	chloranil	none	none	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, polyethylene; 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)	$I_0$ (nA)	Sensitivity (nA/mM)	Relative sensitivity (5.S/ $I_0$ )	Lowest O <sub>2</sub> (mm Hg)	Drift (%/h)
<i>Glucose sensors used in vivo</i>							
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
<i>Glucose sensors used ex vivo</i>							
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
<i>Glucose sensors used in vitro</i>							
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;  $I_0$ , background current (current at zero glucose); relative sensitivity, response at 5 mM glucose/ $I_0$ ; Lowest O<sub>2</sub>, 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  $I_0$  (Table 3). This  $5 \times S/I_0$  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,  $\gamma$ -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
<i>In vivo Characteristics</i>							
1	rat	none	brain	gen	integral	4	non port
2	dog, rat	none	s.c.	local	integral	0	tel, port
3	rat, dog, rabbit	GA	s.c., i.v.	gen, none	surface	2	tel, port
4	man, sheep	GA	s.c.	local	integral	1	non port
5	rat	gamma	s.c.	gen	integral	0.5	non port
6	rat	EtO	s.c.	gen	integral	0.45	non port
7	man	none	s.c.	local	surface	1	non port
8	man	none	s.c.	local	surface	2	portable
9	dog, rat	EtO, thiomersal	s.c./i.p.	gen	integ./surf.	0.5	portable
10	man	gamma, EtOH	s.c.	none	integral	0.75	tel, port
11	rat	gamma, IPA	s.c./i.v.	gen	integral		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.

Table 5. Operating characteristics of *in vivo* and *ex vivo* glucose sensors

No.	<i>In vivo</i> performance			Explanted sensor performance			Calibration	No. points
	Duration (d)	Sensitivity (nA/mM)	S <i>in vivo</i> /S <i>in vitro</i> (%)	S/S <i>in vitro</i> (%)	T/T <i>in vitro</i> (%)	L/L <i>in vitro</i> (%)		
<i>In vivo</i> 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 <sup>a</sup>	100	100	100	100	100	100	100	100	1
13	30	1800	900	100	100	100	100	100	100	100	—
14	150	1.0	100	100	100	100	100	100	100	100	2
15	3	1.2	100	95	105	100	100	100	100	100	1
16	1	8.5	100	100	100	100	100	100	100	100	2
Mean	37	453	260	99	101	101	100	100	100	100	
SD	58	778	320	2	2	5	0	0	5	0	
No	5	4	5	5	5	5	5	5	5	5	
Min	0.3	1	100	95	100	100	100	100	100	100	
Max	150	1800	900	100	105	105	100	100	100	100	

Key:

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

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.

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