PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Microelectrodes in Medicine [and Discussion]

I. A. Silver and I. Bergman

Phil. Trans. R. Soc. Lond. B 1987 **316**, 161-167
doi: 10.1098/rstb.1987.0025

Phil. Trans. R. Soc. Lond. B **316**, 161–167 (1987) [161]
Printed in Great Britain

Microelectrodes in medicine

By I. A. Silver
Department of Pathology, Medical School, University of Bristol, Bristol BS8 1TD, U.K.

The chief applications of microelectrodes in medicine are in experimental studies of tissue and cell behaviour and of metabolism. Microprobes have been developed for a wide range of specific substrates and for the detection of bioelectric potentials. They have provided information that now forms the basis of much of our understanding of biological phenomena. Their inherent characteristics, such as fragility and 'drift', render them unsuitable for general clinical application except at the surface of organs. The heterogeneity of tissues makes it necessary to specify the exact anatomical location of microelectrode measurements and the probes themselves must be of a form that neither distorts, damages, nor stimulates reactions from, the cells or blood vessels with which they are in contact.

Introduction

The major application of microelectrodes in biomedical science is in the direct measurement of conditions in tissues; a second and less demanding requirement is for systems that will monitor specific substances or a range of substances in biological fluids. The definition of what constitutes a microelectrode is elusive. For some it is any sensing device that has a very small reactive area (in the range of nanometres to micrometres), regardless of its overall dimensions; for others, the small detector must be carried on a probe with an almost equally small, and usually sharply pointed, tip.

Some of the special problems associated with microelectrodes used in living organs are caused by the anatomical structure of tissue and in particular its heterogeneity. At the gross level the most obvious structural differences are those between mineralized hard tissue, such as bone, and the soft tissues, whereas at the cellular level quite major differences in physical conditions exist on either side of the plasmalemma. Probes for tissue monitoring must be of a shape and size that do not distort or damage what is being measured and made of materials that are biocompatible and do not provoke inflammatory reactions over the time course of the proposed measurements. Microelectrodes therefore fall essentially into three categories: (i) devices with a small detector area in a robust carrier, such as intravascular catheters or tissue surface electrodes (Fatt 1964); (ii) probes that are small enough and suitably shaped to penetrate tissue and cause minimal damage, but which measure only extracellular conditions; (iii) electrodes with tip sizes of the order of 0.1–0.5 μm that can be used for intracellular measurements.

Any micromonitoring device must be capable of use in such a way that it does not compromise local blood supply at the capillary level either by physical compression or by stimulating changes in flow, and its functional characteristics must not be such as to cause enhanced or depressed metabolism or trigger electrical activity.

Voltage-sensitive microelectrodes

The application of microsensors to the study of biomedical problems probably began with the introduction of the voltage-sensitive, electrolyte-filled glass micropipette for the measurement of cell-membrane characteristics. Initially, hand-pulled pipettes were applied to the investigation of very large cells, such as the giant nerve axons of some cephalopods, particularly *Sepia*. The use of simple voltage-sensitive microprobes allied to elegant experimental techniques led to the elucidation of the biophysics of conduction of nerve impulses.

Progression from hand-pulled to machine-fabricated glass micropipettes greatly improved the reproducibility of their shape and the achievement of the very small tip size necessary for penetrating mammalian cells without causing unacceptable damage to the plasmalemma. The application of these probes to the investigation of the physiology of electrically active and other cells has provided much of our present knowledge of cell behaviour.

A later development in microelectrode technology was the production of metal-based probes with tips of the same order of size as the glass microcapillaries (0.1–2.0 μm). These were either made from very robust metal, such as tungsten, stainless steel, platinum (Ballantijn 1961), or platinum–iridium (Silver 1965), insulated with resin or glass or were metal-filled glass micropipettes (Whalen *et al.* 1967). They were primarily used for extracellular recording of nerve action potentials, but the noble-metal probes formed the basis for micropolarographic electrodes (Silver 1965; Lubbers & Baumgartl 1967; Whalen *et al.* 1967).

Microsensors for oxygen

Davies & Brink (1942) applied amperometry to the measurement of oxygen tension in the brains of cats and introduced the first true substrate-specific microelectrodes into biomedical research. They examined the problems of measuring oxygen in tissue and concluded that only 'recessed' electrodes, subjected to intermittent voltage pulses, gave absolute values for oxygen tension in tissues. They also demonstrated that continuously polarized 'open-tipped' (non-recessed) electrodes, although more capricious than the recessed type, were better able to follow the rapid changes in p_{O_2} which often occur in living tissue. Moreover, they showed for the first time that there were steep gradients of oxygen tension in metabolically active tissue such as brain.

The significance to medicine of these observations and techniques was not widely recognized until interest developed in the radiosensitizing effect of oxygen and the possibility of its use in radiotherapy of malignant tumours. Amperometric oxygen cathodes of various sizes and designs were developed for both experimental and clinical investigation of p_{O_2} in tumours. They proved particularly useful for identifying the differential effects on normal and malignant tissues of changes in the oxygen content of inspired gases (Montgomery & Horwitz 1950; Cater *et al.* 1957; Evans & Naylor 1960).

In addition to their role in studies on tumours, oxygen-sensitive microelectrodes have been used in many physiological, pathological and clinical investigations of conditions at the surface of and within a wide variety of tissues. They have been exploited especially in studies on the brain during epileptic seizures, hypoxia and ischaemia and in peripheral organs during shock and other cardiovascular disorders. The concept of the multiwire 'Kammer-Elektrode' (Kunze *et al.* 1962) developed from the need to measure 'average' p_{O_2} over relatively large areas of

exposed tissues during surgery or in intensive-care units, to determine the statistical distribution of the different values. This has proved useful in determining whether an organ suspected of harbouring some pathological condition is or is not adequately oxygenated. Multiple arrays of microelectrodes have the advantage over single large cathodes in that they do not compete to the same extent with the cell population for the available oxygen. Furthermore, the volume of tissue from which they draw oxygen is more easily identified and variations in oxygen availability to the tissue over small distances become apparent.

A major problem in the application of oxygen microelectrodes to tissue measurements is the sensitivity of the probes to (i) micromovements and (ii) 'poisoning' either by deposition of protein or complexing of sulphydryl groups, derived from damaged cells, on the surface of the metal. Many different 'protective' membranes have been used on the surface of microelectrodes to minimize these effects, but none is wholly successful. Miniaturization of the original Clark electrode is tedious and results in a very fragile probe (Silver 1965).

Although Davies & Brink (1942) had shown the feasibility of pulsed voltammetry for measurement of absolute local tissue p_{O_2} with 'recessed' micro- and semimicroelectrodes and advocated the advantages of this technique, most later workers used continuously polarized amperometric electrodes and were more concerned with changes in tissue oxygenation than in determination of absolute values, because the latter vary sharply according to exact anatomical site and are difficult, if not impossible, to interpret. Sweep potential polarography applied to 'open' electrodes in tissues also presented technical electronic problems until relatively recently, and although investigated by Olson *et al.* (1949) and developed for the study of tumour oxygenation by Naylor & Evans (1960) it was applied only sporadically by other workers and remained relatively unpopular until appropriate microcircuits became available (Hahn 1980, 1981; Laycock *et al.* 1984).

Sweep potential polarography has been applied to single and multicathode microelectrode systems for the measurement of local and regional oxygen tension in tissues. The form of the probes has varied from single gold or platinum–iridium needles with a tip diameter of 0.5–2.0 μm to multiple arrays of flat-ended 1–5 μm platinum wire-in-glass systems. The advantage of the intermittent sweep potential is that the use of oxygen by the probe is greatly reduced and thus it is less likely than continuously polarized systems to alter the conditions being measured. This is an important consideration in living tissue, which may respond to the presence of a probe by changes in behaviour and thus vitiate the attempted measurement. Secondly, the determination of absolute oxygen concentration is more easily achieved, especially when short pulses are used. Thirdly, measurements at tissue surfaces can be made very quickly with minimal equilibration time; this speed renders the technique especially appropriate for intra-operative measurements on patients undergoing surgery, when it is often impossible to wait for conventional DC systems to stabilize before reliable readings can be obtained. Finally, an array of microelectrodes distributed over a relatively large area can give a rapid indication of the mean p_{O_2} of the tissue together with its range, and provide clinically useful information on the state of the patient.

In vitro micro- and semimicroelectrodes have been used routinely for many years in clinical blood-gas analysis. The designs derive from the classical membrane-covered Clark macroelectrode (Clark 1956) but the cathodes have been reduced in size to between 5 and 50 μm to minimize oxygen utilization and allow the routine analysis of very small blood samples, as electronic circuits have become more capable of handling small currents.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Hydrogen electrodes

Metal microelectrodes can also be used for the local measurement of capillary blood flow in tissue. Platinum or palladinized needle electrodes, when polarized positive at between 50 and 150 mV, detect molecular hydrogen. Free hydrogen is not present in the blood of mammals (other than some ruminants) in significant amounts, but if it is introduced briefly into the respired gas in very low concentration, its 'washout' time from blood-perfused tissue can be measured with hydrogen-sensitive microelectrodes and the local blood flow rate can be calculated intermittently (Lübbers & Baumgärtl 1967). For continuous measurement of flow, a second, slightly larger electrode is placed in the tissue near the first, and polarized so that hydrogen is generated at its tip. The gas diffuses into the tissue and is detected by the nearby sensor. The amount of hydrogen reaching the latter will depend on the local blood flow rate and direction. Thus, changes in current output of the sensor can be related to percentage changes in capillary blood flow, although quantitative measurements are unlikely. An array of microelectrode sensors may be sited around a generator electrode to provide information on changes in direction of flow in the capillary bed.

Enzyme microelectrodes

Miniaturization of substrate-specific macroelectrodes for use in tissues has not been particularly successful. Glucose-sensitive microprobes have been made by Silver (1976). The design was derived from the glucose oxidase electrode of Clark & Clark (1973), a platinum-black-coated platinum microneedle impregnated with glucose oxidase. The electrode is polarized (600 mV) positive; the reaction at the electrode surface involves reduction of hydrogen peroxide to water. Such electrodes are useful in well-oxygenated tissue, but in practice, despite the low K_m of glucose oxidase for oxygen, the supply of oxygen may become rate-limiting for the enzyme-mediated oxidation of glucose in hypoxic tissue, which is where it is usually important to know the state of glucose supply. The use of artificial electron-transfer systems, such as ferrocene derivatives and the so-called organic metal salts, may overcome this problem, because they permit the oxidation of enzymes in a hypoxic environment. Unfortunately many of these substances are very toxic and hazardous to use in tissue. Tissue microelectrodes polarized at 600 mV will also respond to ascorbic acid and catecholamines; this response can cause difficulties in certain organs such as adrenal gland where there are relatively high concentrations of these interfering substances.

Microelectrode sensors for various amino acids, based on the appropriate oxidases, have not yet proved reliable for *in vivo* measurement of these substances. However, there is no theoretical reason why they should not be developed with present technology.

Microsensors for lactate, based on a lactate dehydrogenase–pyridine nucleotide mixtures, have been the most effective in experimental use but are very capricious. These have had a metal or 'glassy carbon' core (Silver 1977) and are superior to lactate-oxidase-based systems, which are oxygen-sensitive. This latter is a major disadvantage, as lactate is most usually formed in hypoxic tissue. New systems, including those that use conducting organic salts, show promise in overcoming this problem.

A more successful application of polarography to the detection of metabolites is in the measurement of catecholamines with carbon-fibre electrodes. Microsensors of this type have

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

been particularly valuable when used in conjunction with microiontophoretic application of substances, such as neurotransmitters, to precise parts of cells or microregions of the brain. The sensor provides a feedback system for controlling the amount of active material injected. The technique where a multibarrelled microprobe acts both as applicator and sensor is especially useful in the study of substances that may have two or more biological actions depending on their concentration.

Ion-sensitive microelectrodes

The first ion-sensitive semimicroprobe was probably the pH electrode designed by Caldwell (1958) for insertion into the cut ends of invertebrate giant axons. The electrode was approximately 80 μm in diameter and of conventional form. In 1959, Hinke introduced a much smaller, all-glass, ion-sensitive probe design with a tip diameter of 5–10 μm but with a sensitive region at least 20 μm long. This rendered it suitable only for extracellular use in mammalian tissue although it was satisfactory for intracellular investigations in some invertebrate giant cells. A novel design by Thomas (1974) involved the enclosure of a pH-sensitive microprobe in a standard borosilicate glass micropipette, thus shielding the former from mechanical damage and at the same time ensuring that even a minimal penetration of a cell wall would bring only protons from the interior of the cell into contact with the probe. Since the tip size of the outer electrode can be as small as 0.1 μm it is feasible to use this probe for intracellular measurements on mammalian cells, but it has been applied chiefly to investigations on the behaviour of large invertebrate neurons (see Thomas 1984).

Substitution of glass membranes sensitive to ions other than protons has resulted in the production of all-glass microelectrodes specific for sodium and potassium, but neither of these has great selectivity against other ionic species commonly found in tissue. Furthermore, although such electrodes can be used readily for investigations inside large invertebrate cells, in conjunction with a separate intracellular reference electrode, this is not feasible with mammalian cells. Although successful attempts have been made to produce double-barrelled, all-glass, ion-sensitive electrodes (Zeuthen 1976) the technical difficulties are great and render their general use impractical.

Liquid ion-exchanger and neutral-ligand microelectrodes

The problems outlined above in the fabrication and lack of discrimination of some types of all-glass electrode have been circumvented to a large extent by the development of a wide range of liquid ion exchangers (LIX) and neutral ligands with high specificity for individual ions of biological importance. The first application of these substances to microprobes for use in tissue was that of Walker (1971) who rendered a standard glass micropipette hydrophobic by siliconization and filled the tip with the potassium-sensitive LIX Corning 477318 before filling the shaft of the pipette with potassium chloride solution. This microelectrode could be made small enough for intracellular use; more importantly, it was possible to make it relatively easily in a double-barrelled configuration (Khuri *et al.* 1972) and it has therefore become the preferred method for direct measurement of intracellular ion activities. The requirement for double-barrelled electrodes is imposed by the existence of the cell membrane potential, which contributes to the voltage recorded with an intracellular ion-selective electrode. To

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

obtain a true measure of the ion activity it is therefore necessary to know the value of the membrane potential and to subtract it from the output of the ion-exchanger probe.

The original ion-exchangers were neither very selective nor specific. However, Simon and his colleagues have produced a wide range of neutral-ligand ion carriers, which are suitable for use in microelectrodes (Ammann *et al.* 1973, 1981). These include ligands sensitive to calcium, magnesium, potassium, protons and sodium, some of which have very high specificity (Kessler *et al.* 1973). An important feature of liquid sensors for use in microelectrodes is that they must be of relatively low impedance, because microelectrodes have inherently high impedance and if this is greatly increased by the sensor the probe may be unusable. In certain conditions, especially if the ligand may be toxic to cells, it is an advantage for the liquid sensor to be gelled; PVC has proved suitable for this purpose (Alvarez-Leefmans *et al.* 1981). Recent interest in the role of calcium in control of local blood flow and its possible importance in brain damage has increased interest in ion-sensitive microelectrodes for solving important clinical problems.

References

Ammann, D., Pretsch, E. & Simon, W. 1973 Darstellung von neutralen lipophilen Ligande fur Membranelektroden mit selektivitat für Erdalkali-ionen. *Helv. chim. Acta.* **56**, 1780–1791.

Ammann, D., Lanter, F., Steiner, R. A., Schulthess, P., Shijo, Y. & Simon, W. 1981 Neutral carrier based hydrogen ion selective microelectrode for extra- and intracellular studies. *Analyt. Chem.* **53**, 2267–2269.

Alvarez-Leefmans, F. J., Rink, T. J. & Tsein, R. Y. 1981 Free calcium ions in neurones of *Helix aspersa* measured with ion-selective microelectrodes. *J. Physiol., Lond.* **315**, 531–548.

Ballantijn, C. M. 1961 Fine tipped metal microelectrodes with glass insulation. *Experientia* **17**, 523–526.

Caldwell, P. C. 1958 Studies on the internal pH of large muscle and nerve fibres. *J. Physiol., Lond.* **142**, 22–62.

Cater, D. B., Silver, I. A. & Phillips, A. F. 1957 Apparatus and techniques for the measurement of oxidation–reduction potentials, pH and oxygen tension *in vivo*. *Proc. R. Soc. Lond.* B **146**, 289–297.

Clark, L. C. 1956 Monitor and control of blood and tissue oxygen tension. *Trans. Am. Soc. artif. int. Organs* **2**, 41–46.

Clark, L. C. & Clark, E. W. 1973 Differential anodic enzyme polarography for the measurement of glucose. *Adv. exp. Med. Biol.* **37**A, 127–133.

Davies, P. W. & Brink, F. 1942 Microelectrodes for measuring local oxygen tension in animal tissues. *Rev. scient. Instrum.* **13**, 524–533.

Fatt, I. 1964 An ultramicro oxygen electrode. *J. appl. Physiol.* **19**, 326–329.

Hahn, C. E. W. 1980 Techniques for measuring the partial pressures of gases in the blood. Part I – *in vitro* measurements. *J. Phys.* E **13**, 470–482.

Hahn, C. E. W. 1981 Techniques for measuring the partial pressures of gases in the blood. Part II – *in vivo* measurements. *J. Phys.* E **14**, 783–797.

Hinke, J. A. 1959 Glass microelectrodes for measuring intracellular activities of sodium and potassium. *Nature, Lond.* **184**, 1257–1258.

Kessler, M., Hajek, K. & Simon, W. 1976 Four-barrelled microelectrode for the measurement of potassium, sodium and calcium ion activities. In *Ion and enzyme electrodes in biology and medicine* (ed. M. Kessler, L. C. Clark, D. W. Lübbers, I. A. Silver & W. Simon), pp. 136–140. Munchen, Berlin and Wien: Urban and Schwartzenberg.

Khuri, R. N., Hajjar, J. J. & Agulian, S. K. 1972 Measurement of intracellular potassium with liquid ion-exchange microelectrodes. *J. appl. Physiol.* **32**, 419–422.

Kunze, K., Lübbers, D. W. & Windisch, E. 1962 Die Messung des absoluten Sauerstoffdruckes mit der Kammer-Pt-Elektrode in beliebigen Medien, insbesondere im Blut und Gewebe. *Pflügers Arch. ges. Physiol.* **276**, 414–428.

Laycock, J. R. D., Coakham, H. B., Finney, R., Silver, I. A. & Walters, F. J. M. 1984 Sweep potential polarography on sheep brain cortex. *Neurol. Res.* **6**, 69–74.

Lübbers, D. W. & Baumgärtl, H. 1967 Herstellungstechnik von palladinierten Pt-Stich-electroden (1–5 µm Aussendurckmesser) zum polarographischen Messung des Wasserstoffdruckes für die Bestimmung der Micro-zirkulation. *Pflügers Arch. ges. Physiol.* **294**, R 39–40.

Montgomery, H. & Horwitz, O. 1950 Oxygen tension of tissues by the polarographic method. 1. Introduction: Oxygen tension and blood flow of the skin in human extremities. *J. clin. Invest.* **29**, 1120–1126.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Naylor, P. F. D. & Evans, N. T. S. 1960 The measurement of oxygen tension by rapid voltage sweep polarography and bare platinum electrodes. *J. polarogr. Soc.* **2**, 9–25.
Olson, R. A., Brackett, F. S. & Crickard, R. C. 1949 Oxygen tension measurement by the method of time selection using the static platinum electrode with alternating potential. *J. gen. Physiol.* **32**, 681–703.
Silver, I. A. 1965 Some observations on the cerebral cortex with an ultramicro, membrane-covered oxygen electrode. *Med. Electron. biol. Engng* **3**, 377–387.
Silver, I. A. 1976 An ultra-micro glucose electrode. In *Ion and enzyme electrodes in biology and medicine* (ed. M. Kessler, L. C. Clark, D. W. Lübbers, I. A. Silver & W. Simon), pp. 189–192. Munchen, Berlin and Wien: Urban and Schwartzenberg.
Silver, I. A. 1977 Ion fluxes in hypoxic tissues. *Microvasc. Res.* **13**, 409–420.
Thomas, R. C. 1974 Intracellular pH of snail neurones measured with a new pH sensitive glass microelectrode. *J. Physiol., Lond.* **238**, 159–180.
Thomas, R. C. 1984 Experimental displacement of intracellular pH and the mechanism of its subsequent recovery. *J. Physiol., Lond.* **354**, 3*P*–22*P*.
Walker, J. L. 1971 Ion-specific liquid ion exchanger microelectrodes. *Analyt. Chem.* **43**, 89A–93A.
Whalen, W., Riley, J. & Nair, P. 1967 A microelectrode for measuring intracellular P_{O_2}. *J. appl. Physiol.* **23**, 798–802.
Zeuthen, T. 1976 A double-barrelled Na^+-sensitive electrode. *J. Physiol., Lond.* **254**, 8*P*.

Discussion

I. Bergman (*Health and Safety Executive, Broad Lane, Sheffield, U.K.*). Professor Silver has extensive experience of amperometric oxygen sensors, and now he is using phosphorescence-quenching oxygen sensors. I also invented sensors based on both principles, and had to choose between them. We wanted portable instruments that would last six months between servicing and that would be intrinsically safe in flammable gases, so we concentrated on the amperometric systems. We found that the longer-lifetime phosphors tended to fade most rapidly. Could Professor Silver indicate how he sees the two systems developing in his field?

I. A. Silver. I feel that amperometric systems are unlikely to develop much further except in relation to biocompatible membranes that protect the cathodes from 'poisoning' and protein coating. In the area of microprobes for experimental use in tissue, intrinsic chromophores, such as pyridine nucleotides, have been somewhat disappointing, but biocompatible exogenous probes (either microencapsulated or as supravital dyes) allow a non-invasive approach to tissue measurement. In the clinical field the advantages of optical probes for relatively short-term measurements are that there is no electrical hazard to the patient, some systems are completely non-invasive and there is less inherent tendency to drift than in electrodes. The use of 'lifetime' measurements and appropriate phosphors allows considerable loss of chromophore without loss of sensitivity or change of calibration. In my view optical methods are likely to replace most electrode systems for clinical monitoring of patients over the next few years.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES