Meetings

Microelectrodes

Eighteen years have passed since Ling and Gerard [*J. Cell. Comp. Physiol.* **34**, 383 (1949)] reported the direct measurement of membrane potentials with a glass micropipette electrode. The technique was sufficiently attractive and simple to lead many researchers to adopt it.

For many investigators, however, intracellular glass microelectrodes have remained a poorly defined tool with unpredictable behaviors. The absence of an accepted theory of glass microelectrode behavior, with their accomplishments as a most powerful research tool, inspired the organization of a conference, the first of its kind, on microelectrodes in Montreal, Canada, 23–25 May 1967.

Fifteen invited and 14 contributed papers were presented in the 3-day conference. The great interest in this highly specialized research tool was evidenced by the more than 200 persons in attendance.

The program was organized to cover all aspects of glass microelectrodes. It included discussions of inner reference electrodes, microelectrode glasses, and open-tip (micropipettes) and closed-tip microelectrodes (miniaturized dip-type electrodes). It also included discussions of the applications of microelectrodes in studies of cellular membrane properties, in studies of cytoplasmic properties, and in studies of cellular intervention by microinjection techniques.

Inner reference electrodes were discussed by Roger G. Bates. These reference electrodes are a major determinant of the zero potential and temperature coefficient of the complete cell. Serious errors can arise from changes in temperature of the system since the Nernst slope, the standard cell potential, and the *p*H of the unknown are all temperature dependent. Errors can be reduced by measuring the potential of the standard cell at the temperature of the *p*H measurement and selecting solution and inner reference electrode so that the temperature coefficient of the electromotive force for the inner half-cell compensates that of the working electrode.

Properties of microelectrode glasses were summarized by Normand C. Hebert. It is important to know properties of glasses, such as thermal expansion coefficients and softening points, for the sealing of two dissimilar glasses. The softening point of a glass also gives an indication of the coil temperature necessary for pulling microelectrodes. The chemical durability of a glass determines the procedure of filling electrodes. The type of electrode, that is, open-tip or closed-tip, can be determined from specific electrical resistivity data and a knowledge of the geometry of the tip.

The electrochemistry of open-tip glass microelectrodes (micropipettes) was discussed by Fred M. Snell, Marc Lavallée, Daniel P. Agin, Philip B. Hollander, and Stanley Rush. Special consideration was given to methods of abolishing artifacts brought by tip potentials. Fred M. Snell derived equations for the electrical resistance, liquid junction potential, and electrokinetic properties of ultrafine micropipette electrodes (tip diameters in the order of 0.01 micron). The electrical resistance of a given micropipette is directly proportional to the cotangent of its conical included angle and inversely proportional to the tip radius. One can avoid significant junction potentials by using relatively large tips along with electrolytes whose ionic mobilities counteract the ion exchange characteristics.

According to Marc Lavallée surface conduction along the inner glass wall can account for the tip potential and resistance variations of micropipette electrodes made of high-electrical-resistivity glass. The glass electrode function remains important in micropipettes made of a low-resistivity glass. Micropipettes can be utilized as *p*H indicators, since the surface charge density of the glass depends on the hydrogen ion concentration of the surrounding medium. In low-resistivity glass micropipettes, the potentials arising from the glass electrode function of the wall and from the surface conduction of the glass can be added algebraically. It is therefore possible to obtain an electrode potential change larger than 60 mv per *p*H unit at room temperature. This phenomenon is called "ionic amplification."

Daniel P. Agin, in seeking an explanation for the behavior of the glass micropipette electrode, focused his attention on a property of the Stern layer, that is, the reversal of the sign of charge density brought about by the absorption of a cation such as thorium. Agin has found that traces of thorium chloride (10 micromolar) significantly reduce the tip potential. The tip potential is believed to result from an asymmetry between the exposed surfaces of the glass.

Special types of microelectrodes were discussed by Edouard Coraboeuf, Tsuneo Tomita, and Yoshiaki Omura as improvements of the classical Ling and Gerard glass micropipette. Edouard Coraboeuf reviewed the use of double-barreled and floating micropipette electrodes on cardiac fibers and certain other tissues. Coraboeuf discussed suspension systems which are currently used and described a system used in his own laboratory that permits a flexible micropipette to remain in a beating cell for as long as 1 hour. It is important that methods employing two micropipettes or double-barreled micropipette electrodes be used for measurements of the input resistance of a biological system. Methods using only one micropipette generally give high values for the input resistance because the micropipette resistance depends upon the value of the specific resistance of the cytoplasm.

Tsuneo Tomita discussed single and coaxial micropipette electrodes which are used to study the retina. Tomita developed an ultrafine micropipette electrode to improve the penetration. The electrode has a tip diameter of 0.1 micron or less. Coaxial micropipette electrodes are mounted so that the axial distance between the two tips can be varied. The coaxial electrode has the advantage of a lower coupling resistance when compared to the double-barreled micropipette electrode.

Yoshiaki Omura described a flexibly mounted, nonpolarizing glass micropipette. This system allows continuous recording of transmembrane action potentials of single cardiac cells of vigorously beating hearts for more than 1 hour in dogs and rabbits, and for several hours in rats, turtles, and frogs.

The application of microelectrodes in studies of cellular membrane properties was discussed by Gregor A. Kurella, C. F. Hazelwood, Harry Grundfest, Ake P. Vallbo, Raja N. Khuri, Eberhard Frömter, and Berton C. Pressman. Gregor A. Kurella's work on the alga *Nitella* deals with potential gradients between the different parts of the cell, first across the cellulose pectinic wall, then through the cytoplasm, and finally into the central vacuole. Microelectrodes filled with antimony or made of pH-sensitive glass are used to measure the pH of the cell sap of *Nitella flexilis*. Kurella also described his method of microinjection into the central vacuole. He measured the chloride and potassium activity in the cell sap through a microfistula.

Harry Grundfest covered the dynamics of the cell membrane as an electrochemical system. Every theory of a biological membrane must consider these membranes to be heterogeneous systems which are highly permselective and very thin. Grundfest also discussed the mechanisms of activation and inactivation in biological membranes.

Raja N. Khuri presented work dealing with the determination of Na^+, K^+, and pH in single proximal tubules of *Necturus* and rat kidneys with microelectrodes. He reported that, in both species, the activity of Na^+ in the intraluminal fluid was the same as in the blood.

The use of microelectrodes in studies of cytoplasmic properties was discussed by Otto Schanne, P. G. Kostyuk, J. A. M. Hinke, Frank W. Orme, and William J. Whalen. Schanne discussed the two methods used in the measurement of cytoplasmic resistivity. The resistance of a micropipette is a function of the resistivity of the solution in which the tip is immersed. The resistance change is characteristic for a given electrode and can be recorded as a calibration curve. Measurements with calibrated micropipettes are subject to the same conditions as measurements of membrane potential. The ionic specificity and pH sensitivity of micropipettes must be carefully controlled in these measurements.

P. G. Kostyuk described a technique for the measurement of hydrogen, sodium, and potassium ions in striated muscle fibers and nerve cells with sealed glass microelectrodes. It was concluded that activity coefficients obtained with ion selective microelectrodes cannot give the true physical nature of the processes of interaction of ions with the structural components of protoplasm. Consequently, the technique cannot be used to determine the degree to which ions are bound.

J. A. M. Hinke has used closed-tipped glass microelectrodes with diameters up to 30 microns and has confined his studies to large cells. From his work with isolated barnacle muscle fibers (*Balanus nubilus*), he concludes that significant quantities of cellular monovalent cations are not free in solution. Moreover, sodium ions and not potassium ions are preferred by intracellular binding sites.

Frank W. Orme described a method of preparing calcium ion and chloride ion microelectrodes. The procedure consists of filling the tips of micropipettes with a liquid organic ion exchanger. Electrodes give near Nernstian response to calcium down to 10^{-5} molar. The chloride electrodes have much lower electrical resistances and are fast responding.

Whalen reported a very fast responding (less than 1 second) pO_2 microelectrode having a tip diameter of about 1 micron, which is not affected by stirring. Such electrodes have been successfully used in studies dealing with cell respiration.

Microinjection techniques were discussed by T. K. Chowdhury and Louis J. DeFelice. Chowdhury presented a new method for the microinjection of electrolytes and nonelectrolytes into a microlocus within an individual cell or tissue. The method utilizes a high-frequency axial vibration imposed on a micropipette filled with the desired solution.

The conference was sponsored by Corning Glass Works with the cooperation of the University of Sherbrooke. Abstracts of papers can be obtained gratis from Corning Glass Works, Technical Information Center, Sullivan Park, Corning, New York 14830.

NORMAND C. HEBERT
Corning Glass Works, Corning, New York

MARC LAVALLÉE
OTTO SCHANNE
Department of Biophysics, University of Sherbrooke, Québec

Calendar of Events

Courses

Application of Computers and Systems Modeling to the Solution of Power System Problems, University of Toronto, 6–17 May. The course is intended for power system engineers, system planners, applied mathematicians, and educators. It is anticipated that this course will (i) familiarize the participants with the organization, algorithms, programming, and simulation techniques associated with the digital computer; (ii) provide the required background of numerical and mathematical methods applicable to the use of digital equipment for active control and problem solving; (iii) allow evaluation of system models and computational economy when applied to the solution of system transient, planning, control, and economic problems. Fee: $350. (Division of Extension, Business and Professional Courses, University of Toronto, 84 Queen's Park, Toronto 5, Ontario, Canada)

Composite Materials: Fundamentals and Utilization, University of California, 14–19 June. The course will cover advances in composite materials and structures technology and will explore the potential structural applications of composites. Aspects of research and technology to be discussed include micro and macro mechanisms, nonlinear elasticity, structural design, anisotropy of fracture and strength, experimental techniques employed in fracture studies and directional solidified metals and ceramics. Fee: $260. (Engineering Extension, University of California, 2223 Fulton St., Berkeley 94720)

Grants and Fellowships

Medical Research. The Life Insurance Medical Research Fund has announced grants-in-aid of medical research to become effective 1 July 1969. These grants are made to nonprofit institutions for support of basic research in physiology, biochemistry, and other fields related to medicine. *Deadline for receipt of applications: 15 September.*

The Fund also offers medical scientist fellowships to medical students willing to prepare for careers in teaching and research by securing both the M.D., and the Ph.D. or its equivalent. The fellowships offer a maximum of 6 years of aid; fellowships may be activated at various stages of the M.D.–Ph.D. training. Each school of medicine is invited to make two nominations for aid to begin 1 July 1969. *Deadline for receipt of applications: 15 October.* (Scientific Director, Life Insurance Medical Research Fund, 1030 East Lancaster Ave., Rosemont, Pa. 19010)

Oncology. The Cancer Section of the Oklahoma Medical Research Foundation is offering fellowships in oncology to interested persons holding either the M.D. or the Ph.D. degree. Clinical studies include experimental therapy, clinical pharmacology of chemotherapeutic agents, studies of the natural history, biology, and biochemistry of human cancer. Programs can be elected leading either to the practice of clinical oncology or to clinical research in oncology. Nonclinical studies include experimental therapy of rodent tumors, pharmacology and mechanism of action of chemotherapeutic agents, radiation biology, carcinogenesis, regulatory processes in cells growing in culture, cytogenetics, and electron microscopy. Stipends are commensurate with training and experience. (Dr. Paul T. Condit, Head, Cancer Section, Oklahoma Medical Research Foundation, 825 Northeast 13 St., Oklahoma City 73104)