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The glass micropipette electrode: A history of its inventors and users to 1950

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Soon after the glass micropipette was invented as a micro-tool for manipulation of single bacteria and the microinjection and microsurgery of living cells, it was seen to hold promise as a microelectrode to stimulate individual cells electrically and to study electrical potentials in them. Initial successes and accurate mechanistic explanations of the results were achieved in giant plant cells in the 1920s. Long known surface electrical activity in nerves and muscles was only resolved at a similar cellular level in the 1930s and 1940s after the discovery of giant nerve fibers and the development of finer tipped microelectrodes for normal-sized cells.

It is commonly, but erroneously, supposed that sharp glass micropipette electrodes were co-invented by Gilbert Ning Ling and Ralph Waldo Gerard (Ling and Gerard, 1949; much as the invention of the ubiquitous "Pasteur pipette" is incorrectly attributed to Louis Pasteur). Actually, fine, sharp-tipped examples of capillary glass microelectrodes had been developed and used successfully from the 1920s, mainly in plant cells (Bretag, 1983, 2003). Gerard was, however, involved in the successful transfer of their use to single skeletal muscle cells, although his participation in that, too, occurred long before his much-cited 1949 paper with Ling. These aspects of the history of the glass micropipette electrode seem to have been forgotten, accidentally or deliberately.

Very narrow glass tubes aroused scientific interest when capillary action was first noticed in them as a curiosity in around 1660. Robert Boyle writes of "an odde kinde of siphon that I causd to be made a pretty while ago" (Boyle, 1660). He states that examples of slender and perforated "Pipes of Glass" had earlier been given to him by "An eminent Mathematician" who relayed the observations of "some inquisitive French Men (whose Names I know not)" that, when one end was dipped into water, it would "ascend to some height in the Pipe." An explanation for this phenomenon was provided by Robert Hooke, who also reiterated Boyle's version of the history of the small glass pipes (Hooke, 1661). It is pertinent that, soon afterward, Henry Power wrote a book chapter on the subject (Power, 1664) in which he says that he used glass tubes "almost as small as Hairs, or as Art could make them" and named them "Capillary Tubes."

Fine glass pipettes, filamentous glass loops and needles, and the first mechanical devices necessary for their manipulation were developed in the 19th century by Toldt in Germany (1869), Chabry in France (1887), and Schouten in the Netherlands (1899), among others (as reviewed by Chambers, 1918, 1922; Taylor, 1920; Péterfi, 1923). These refined glass instruments succeeded the earlier manufacture of glass tubes (as eyedroppers, medicine droppers, and ink fillers), glass needles, and decorative glass filaments that had, in many cases, dated back through the Renaissance to Roman times.

Eventually, at the beginning of the 20th century, the method of preparing glass capillary micropipettes with tips that proved fine enough to capture a single bacterium was invented by the bacteriologist Marshall Albert Barber (Fig. 1) of the University of Kansas (Barber, 1904). Capillary tubing of hard or soft glass a few millimeters in diameter was held and heated over a microburner until the glass began to soften (as shown in Fig. 2). The hand holding the capillary with forceps was then pulled quickly away horizontally until the rapidly narrowing, and cooling, glass capillary thread, now outside the flame, separated with a slight tug (Barber, 1911). Barber also invented micromanipulators with the three-dimensional precision essential to handle these delicate instruments (Fig. 3), and so to allow them to be inserted through the plasma membranes of living cells without significantly damaging them. In this way, substances or even a single bacterium could be inoculated into the cytoplasm of a living cell, or fluids or structures could be extracted from the cell (Barber, 1914).

Barber's methods were soon noticed by German Nobel Laureate Heinrich Hermann Robert Koch, who subsequently visited the United States in 1908 and observed a demonstration by Barber at the Sixth International Congress on Tuberculosis in Washington, DC (KU History, 2016). Albert Prescott Mathews, Professor of Physiological Chemistry in the Department of Physiology at the

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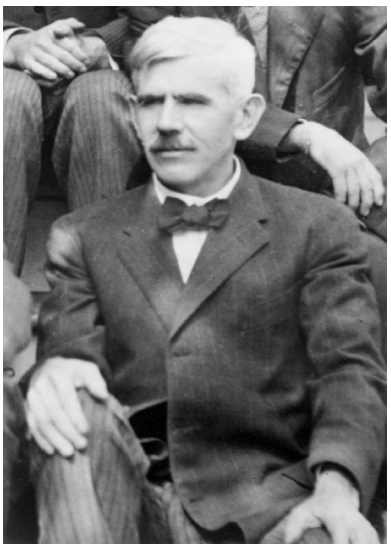

Figure 1. **Marshall Albert Barber (circa 1911).** Image courtesy of the University of Kansas Medical Center Archives.

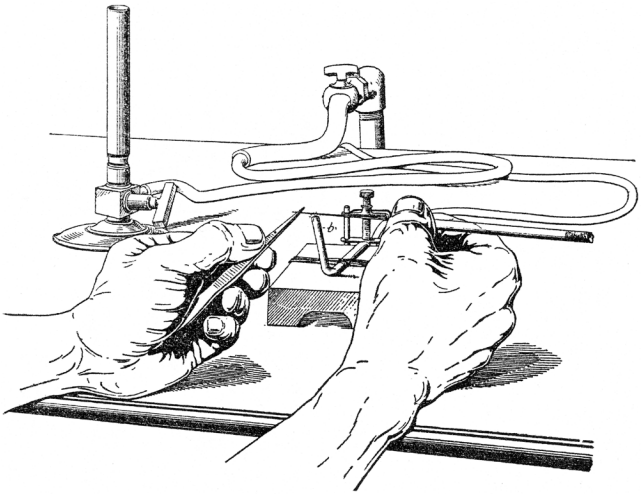

Figure 2. **Barber method of pulling glass micropipettes.** From the *Philippine Journal of Science* (Fig. 5 in Barber, 1914).

University of Chicago, was also aware of Barber's work and sent Research Fellow George Lester Kite to Kansas to learn the micropipette technique in about 1912 (Terreros and Grantham, 1982; Korzh and Strähle, 2002). As we shall see, these are not the last times that the University of Kansas and the Physiology Department at the University of Chicago feature in the micropipette story.

Barber's methods were immediately taken back to Chicago by Kite and, by the summer of 1912, in the seminar series at the Marine Biological Laboratory (MBL) in Woods Hole, he was demonstrating that Barber glass micro-tools could interfere with the development of marine ova. According to Zweifach and Clowes (1958), Kite's lecture greatly stimulated Robert Chambers (Fig. 4), and for a time, these two collaborated (Kite and Chambers, 1912) and championed Barber's techniques. Just how much sophistication had been achieved in preparing micropipettes can be seen from Kite's papers, which mention "needles" of "less than one half micron" in size pulled "from very hard Jena glass tubing about 5mm. in diameter" (Kite, 1912, 1913, 1915).

For the specific purpose of electrically stimulating individual frog sartorius muscle fibers, a very different

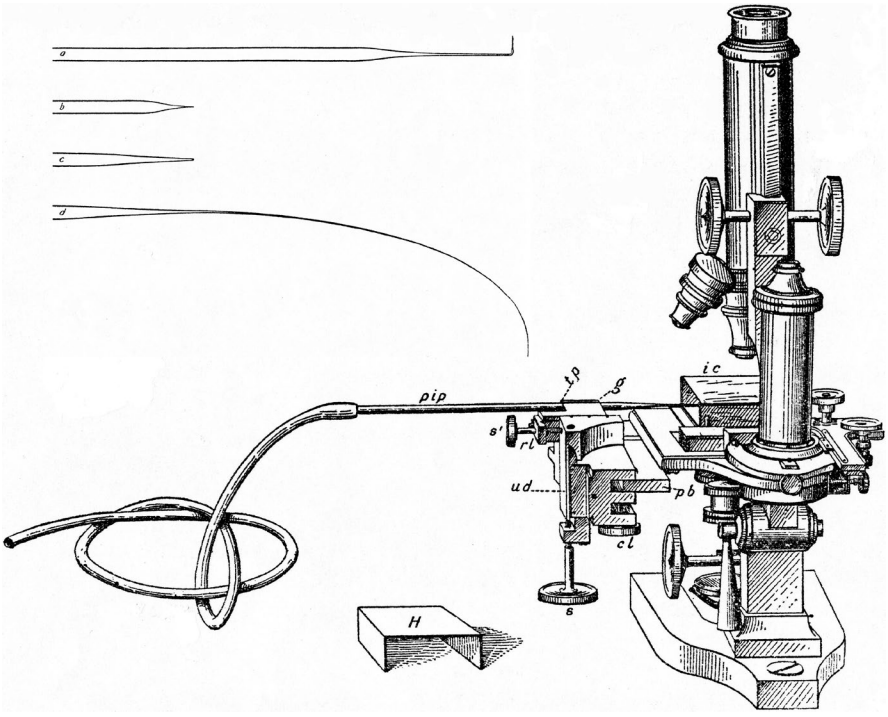

Figure 3. **Barber micropipettes and micromanipulator.** Modified from the *Philippine Journal of Science* (Figs. 1 and 6 in Barber, 1914).

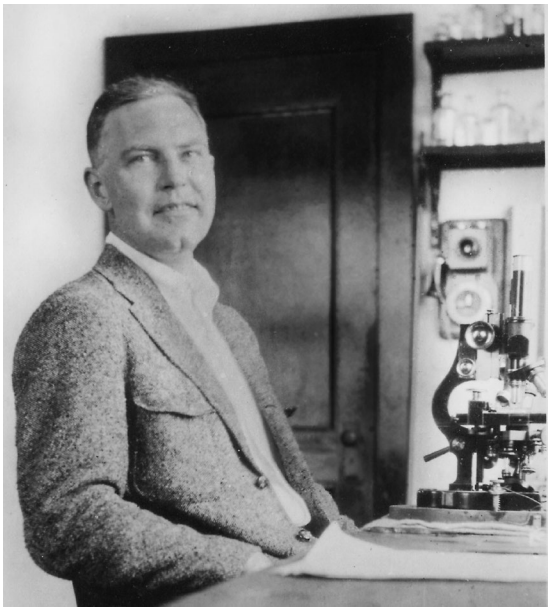

Figure 4. **Robert Chambers (1922).** Image courtesy of the Marine Biological Laboratory, Woods Hole.

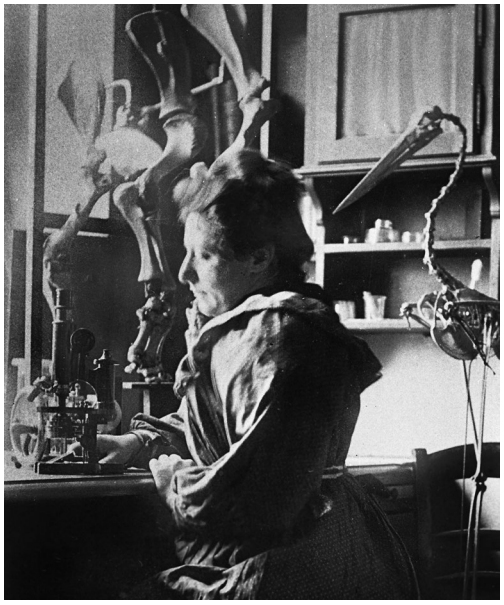

Figure 5. **Ida Henrietta Hyde in her laboratory in Heidelberg, Germany (circa 1896).** Image courtesy of the Spencer Research Library, University of Kansas.

blunt glass pipette was produced by Frederick Haven Pratt (1917) in the form of a concentric "capillary pore electrode." For the central active electrode, Pratt ground back and polished the broad end of a sealed, elongated, capillary tube until a micropore opening with a diameter of 8 µm (and down to 4 µm in one example) appeared. Around this, a concentric, ground glass, leakage junction formed the indifferent electrode. The central active chamber and surrounding indifferent chamber were each filled with NaCl or Ringer solution, attached to the electrical circuitry by nonpolarizing $Zn/ZnSO_4$ junctions. This electrode was used to stimulate individual frog sartorius muscle fibers from which Pratt demonstrated to his satisfaction that, despite graded electrical stimulation, the contractile response of a single muscle fiber was "All-or-None." In his paper, Pratt (1917) described the ease with which the lumen of his electrode could be perfused using an even narrower capillary pipette that would enable the application of chemicals to a restricted area of cell surface and suggested the possibility of iontophoresis. Pratt promoted the use of his reliable and reusable blunt electrode for surface stimulation because "the difficulty of maintenance is vastly less than in the case of a true capillary tube, which is impracticable for this purpose, owing to the excessive electrical resistance and liability to penetrate the tissue, as well as to inevitable serious plugging." This statement suggests that he had already tried and abandoned using Barber-type micropipettes for electrical stimulation.

Nevertheless, Barber micropipettes and micromanipulators were now being adopted, modified, and improved for a variety of intracellular microsurgical and micropipetting purposes (e.g. Chambers, 1918, 1922; Taylor, 1920) and, in a further development, Ida Henrietta Hyde (Fig. 5) prepared and used mercury-filled Barber micropipettes that allowed the electrical stimulation of living cells (Hyde, 1921).

Current flow through her pipettes moved the mercury meniscus toward or away from the tip of the pipette, so enabling expulsion of fluids or their withdrawal into the pipette. Electrical stimulation could be achieved by attaching the appropriate circuitry to the active and indifferent electrodes again through $Zn/ZnSO_4$ junctions. Using this apparatus, she showed that contractions of the stalk of *Vorticella* (Fig. 6) were graded depending on stimulus strength and were not "All-or-None" in contrast to what had been proven by Pratt (1917) for individual muscle fibers. Hyde indicated that although her apparatus "was only in the process of being perfected, nevertheless with it, fluid could be injected and the membrane and other parts of Echinoderm eggs extracted, and these as well as unicellular organisms electrically stimulated." Her main purpose in the publication was to describe multiple possible variations in the construction of micropipette electrodes and to suggest various uses for them. It is uncertain whether she ever proceeded to insert her stimulating electrodes into a *Vorticella* or any other cell body, despite misunderstandings to the contrary (e.g., Tucker, 1981). In her paper, Hyde (1921) claimed no more than that she could stimulate any part of *Vorticella* that was "near contact with the active electrode." In any case, her electrodes were much too large (lumen diameter 3–4 µm) to be inserted into a *Vorticella* stalk

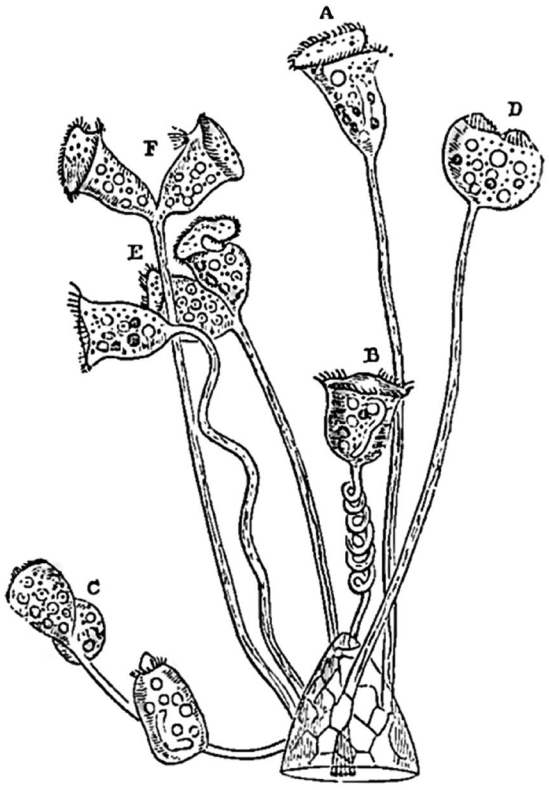

Figure 6. ***Vorticella nebulifera*, showing an ordinary cell with extended stalk at A, another with its stalk contracted at B, and other cells at various stages of fissiparous reproduction.** From Fig. 537 in Carpenter and Dallinger (1891).

(external diameter 2–8 µm). Hyde was, nevertheless, a true pioneer who endured and overcame significant discrimination as a woman in a field dominated by men. After a torturous pathway to become the first woman to obtain a Doctorate in Natural Sciences (the German "Dr. rer. nat." and equivalent of a PhD) from Heidelberg University in 1896 and working at the prestigious Marine Biological Laboratory in Naples, as well as at the Universities of Bern and Harvard, she established the Department of Physiology at the University of Kansas in 1899 (Johnson, 1981; Tucker, 1981). In this last respect, it is not surprising that she developed her electrode from a Barber pipette as we can presume that she must have known Barber who, until 1911, was teaching and undertaking research in Bacteriology and Botany in the same institution. Hyde completed a distinguished career at the University of Kansas, especially championing the cause of women in science, but at about the time of the preliminary report on her Barber pipette microelectrode (Hyde, 1921), she retired and published no more on the subject.

It seems, therefore, that the idea of using sharp micropipettes for extracellular electrical stimulation of cells arose twice, being tested successfully by Hyde after having been discarded as impractical by Pratt. The possibility that micropipette electrodes might be inserted into cells for intracellular electrical stimulation may also have evolved independently more than once, firstly, as (perhaps) hinted at by Hyde, and secondly, more convincingly, by Tibor Péterfi (Fig. 7).

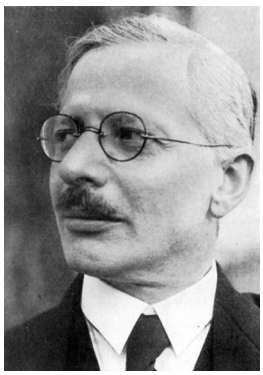

Figure 7. **Tibor Péterfi.** From Chambers and Maskar (1953). Image courtesy of S. Karger, AG, Basel.

After working at universities in Cluj, Budapest, and Prague and serving as a medical officer in WW1, Péterfi had also been preparing Barber-styled micropipettes (Péterfi, 1923) and making improvements to the design of Barber's micromanipulator (using an example obtained from Jacobus Janse, Professor of Botany in Leiden). He had first moved to the University of Jena where he also began work for the Carl Zeiss optical company (Chambers and Maskar, 1953), and Carl Zeiss manufactured the new instrument (Fig. 8) according to Péterfi's instructions. This equipment and accessories, along with a description of experiments and an indication that he had, possibly, already inserted a current-passing micropipette electrode into *Amoeba*, are described in detail in his major work (Péterfi, 1923). In this paper, Péterfi describes the preparation of Jena or Thuringia glass micropipettes barely 1 µm across and ending as a cone rather than as a narrow, tapering "thorn" (= spike), the former overcoming problems of high resistance. Much later, the first definite use of intracellular, current-passing, fluid-filled electrodes occurred in *Valonia* (Blinks, 1930), although these were hardly micropipettes, being capillaries of 0.2–0.5 mm in diameter.

From 1921, Péterfi was at the Kaiser-Wilhelm-Institute für Biologie in Berlin-Dahlem, where he remained until 1934, but he continued to move between laboratories frequently throughout his career (Chambers and Maskar, 1953). Publications from around this time, and later, indicate that Barber, Chambers, and Charles Vincent Taylor (Fig. 9) in the United States and Péterfi in Europe held each other's technical and scientific expertise in high esteem while examples of equipment (including that via Janse and another from Barber to Robert Koch, according to Korzh and Strähle [2002])

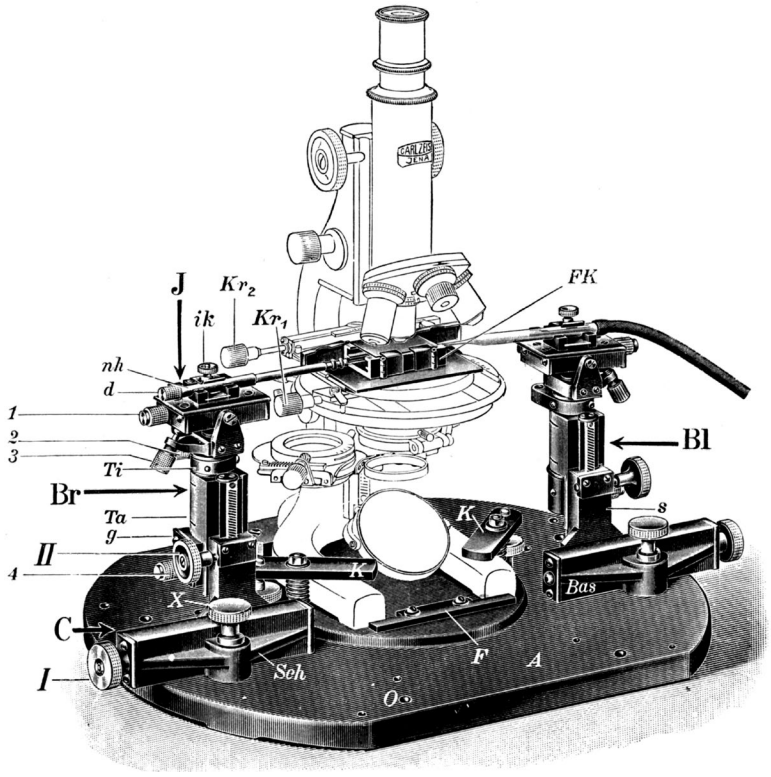

Figure 8. **Janse–Péterfi Micromanipulator (1927).** Image courtesy of ZEISS Archives.

passed each way between them. In his obituary of Péterfi, Robert Chambers (Chambers and Maskar, 1953) noted that the perfected Péterfi-Micromanipulator, manufactured by Carl Zeiss, had become the most widespread instrument of its kind in the world. In this respect, Chambers was being generous. He, too, had invented a popular and widely used micromanipulator (Chambers, 1922) that was later manufactured by Carl Zeiss's competitor, Ernst Leitz, until at least the 1960s. Sophisticated instruments of this kind had become an absolute requirement for the rapidly advancing contemporary research involving micropipettes.

In contrast to the decline in interest in intracellular electrical stimulation after Péterfi (and possibly Hyde), intracellular electrical potential measurement soon obsessed researchers in several laboratories. Initially, these were disappointing and success was very limited. In 1923, Winthrop John Vanleuven Osterhout (Fig. 10) of Harvard University (and later of The Rockefeller Institute for Medical Research) attempted measurements using a submillimeter diameter glass capillary electrode in *Valonia* (Osterhout, 1931). These experiments, conducted at the Bermuda Biological Station for Research, were disappointing as he found a potential difference of only 1–2 mV between the cell sap and the surrounding sea water (Osterhout, 1925). Also, an apparently frustrated Kenneth Stewart Cole says that, in 1924 (Cole, 1968), he "chased paramecia with a pipette at the end of a Compton electrometer" without success (Cole, 1957). By 1925, Péterfi seems to have been the first to measure a variable, but mainly negative, cellular membrane potential in *Amoeba* using a micropipette electrode, although, evidently, he did not seem sufficiently persuaded of the existence of the potential difference to continue to study it (Gicklhorn and Umrath, 1928). It is not surprising that there was confusion. Membrane potentials in *Amoeba* range widely, both positive and negative, depending on motility (or rest)

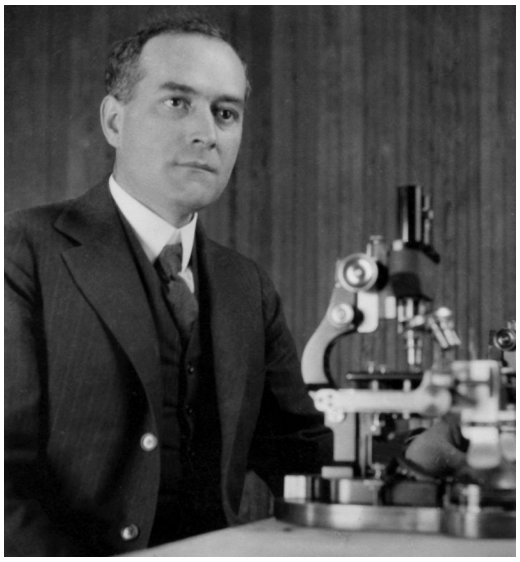

Figure 9. **Charles Vincent Taylor.** Image courtesy of the Marine Biological Laboratory, Woods Hole.

and shape, as well as the composition and pH of their surrounding medium (Braatz-Schade et al., 1973).

From experiments undertaken at Tortugas Laboratory in Florida, Taylor and Douglas Merritt Whitaker, initially, also described only tiny and variable potential differences (Taylor and Whitaker, 1926) between the cell interior and seawater in *Valonia* and in *Clypeaster* eggs (averaging about 2 mV and −1 mV, respectively). Rather than discouraging them, however, they subsequently wrote that the "variability in the results of our use of microelectrodes" (from their 1926 study and from some later, apparently unpublished, work) indicated that better microelectrodes would be indispensable (Taylor and Whitaker, 1927). At Stanford University, Taylor had been perfecting his production of Barber glass micropipettes (Taylor, 1925a) with ultrafine platinum/platinum black inserts to make active (hydrogen) electrodes for intracellular pH estimation or filled with agar-KCl solutions to use as reference electrodes. He described pipettes with tips "having a lumen of even less than 1 micron in diameter" (Taylor, 1925b).

Soon afterward, Taylor and Whitaker (1927) used their improved microelectrodes, not to determine transmembrane potentials, but for pH measurements in *Nitella*. On insertion of their sharpened platinum/platinum black electrode along with their sharp reference electrode, protoplasmic streaming ceased, although it resumed within a minute or so and continued for times up to days with both electrodes still in place, suggesting that impaled cells remained viable. Potentiometric measurements then indicated that in protoplasm, itself, pH could not be determined because of buffer action on the active hydrogen electrode. For vacuolar cell sap that was protoplasm free, however, the pH was found to be as high as 6.1, although even slight contamination by protoplasm lowered its apparent pH.

It was in the giant plant cells that intracellular potentials were first determined successfully using capillary salt bridges and micropipette electrodes in the late 1920s and early 1930s (Osterhout, 1931). *Valonia*, the marine alga, was the earliest to be assessed and found to have an inside positive potential of 5 mV when bathed in sea water (Osterhout et al., 1927). This positive internal potential later proved to be a feature unique of *Valonia* and closely related algae. In these experiments, cells were impaled on cell sap–filled capillaries of 0.2–0.5 mm in diameter (see, e.g., Blinks, 1930). Following Péterfi's methodology, much finer glass micropipette electrodes (as small as 8 µm, but typically 15–30 µm in outer diameter) were used by Josef Gicklhorn and Karl Umrath (Fig. 11) at the German University in Prague to measure potentials averaging −15 mV in *Tulipa* pollen sprouts and −3 to −19 mV in *Nitella* (Gicklhorn and Umrath, 1928). Meanwhile, Samuel Gelfan (Fig. 12) at the University of California, Berkeley, was producing quartz micropipette electrodes of 1–2 µm in tip diameter with which he explored small intracellular potential differences between two separate points in the cytoplasm of *Nitella* (Gelfan, 1927, 1928). In regards to this work, Gelfan acknowledged advice and criticism received from, among others, "Professor C.V. Taylor of Stanford University." Shortly afterward, much more substantial differences of up to 40 mV (but positive inside with respect to outside) were reported between one microelectrode located internally in *Nitella* and another in the immediately adjacent external tap water (Brooks and Gelfan, 1928). Although the stated direction of this potential difference in *Nitella* was soon queried (Umrath, 1930), it seems never to have been satisfactorily explained and the statement, "the electrode within the cell was positive to that outside," may simply have been a mistake. Consistent with much more recent measurements, Umrath (1930) found potential differences in *Nitella* ranging to −164 mV (inside with respect to outside zero) and similar to those mentioned by Osterhout (1931). Within this same period, Blinks (Blinks, 1929, 1930), Damon (1929), and others (see Osterhout, 1931) were able to measure membrane potential differences with larger diameter capillary electrodes in *Valonia*, *Chara*, and *Halicystis* under a variety of experimental conditions.

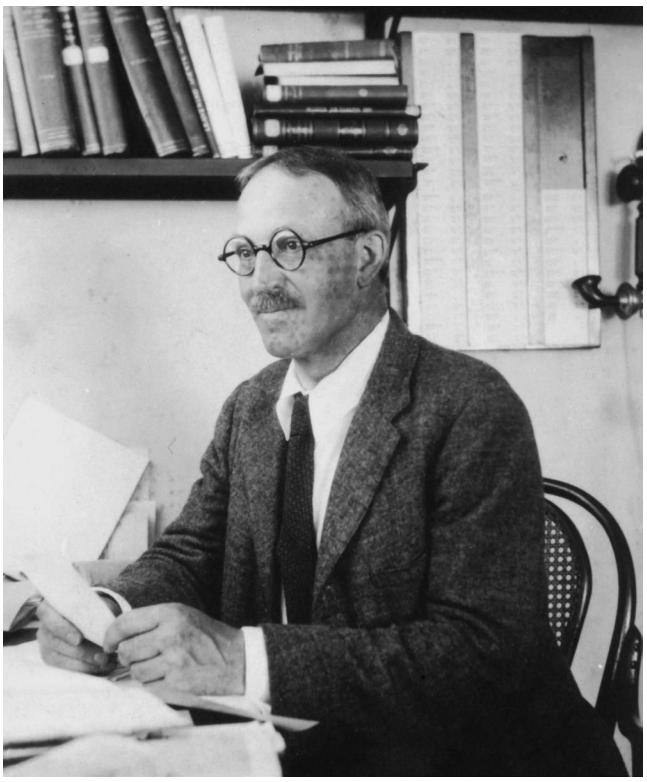

Figure 10. **Winthrop John Vanleuven Osterhout (1922).** Image courtesy of the Marine Biological Laboratory, Woods Hole.

Out of the work from this period, it is my opinion that the contribution of Taylor and Whitaker (1927)

Figure 11. **Karl Umrath.** Image courtesy of the Institute of Plant Sciences, Graz.

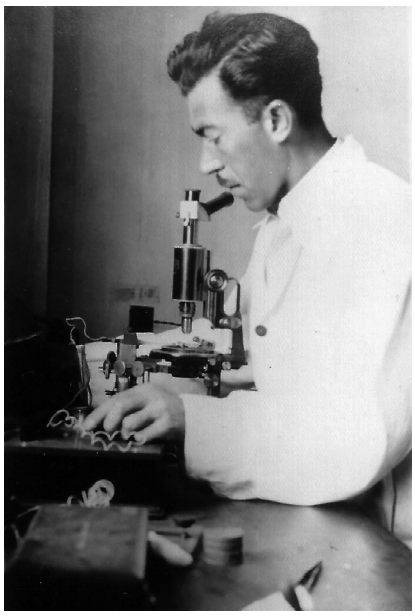

Figure 12. **Samuel Gelfan with his stimulating electrode (circa 1930).** Image courtesy of Carrie Gelfan, from the Gelfan Family Collection. Carrie Gelfan is a daughter of Samuel Gelfan.

presents us with the definitive invention of the intracellular glass micropipette electrode. For their intracellular reference electrodes in this particular study, they had used sharp, agar-saturated KCl-filled Barber micropipettes. By using saturated KCl rather than Ringer solution or low concentrations of NaCl or KCl, as others had done, they greatly reduced the internal resistance of their electrodes and largely eliminated the junction potential between their electrodes and whatever solution they were inserted into, e.g. sea water, pond water, cell sap, or protoplasm. They also used $Ag/AgCl_2$ nonpolarizable junctions for connection to their electrical circuits. It seems quite strange that this remarkable combination of features was neither emphasized by Taylor and Whitaker in their paper nor utilized by others for more than 20 years.

We soon return again, inexorably, to the University of Chicago where Gerard (Fig. 13) was offered an appointment in Mathews' former Department of Physiology in 1928, and at about the same time, Gelfan was awarded a Laura Thorne Donnelley Research Fellowship in the same department. There, and over several summers at the Marine Biological Laboratory, Woods Hole, Gelfan switched to studying animal cells. Notably, he and Gerard spent the summer of 1930 at Woods Hole (The Collecting Net, 1930) and appear to have collaborated in a study using the microelectrodes Gelfan had developed. In this regard, the summary of a seminar on "The All-or-None Law in Muscular Contractions" presented by Gelfan, on July 25, 1930, includes the statement, "work completed in the laboratory at Woods Hole by Dr. Gerard and myself" (Gelfan, 1930a). Transcript of the discussion that followed Gelfan's seminar shows that Gerard was in attendance and that both he and Gelfan answered questions (Gelfan, 1930a). Their study involved the application of small extracellular stimulating voltages to single skeletal muscle fibers, with graded contractions in microscopic percentages of the length of a fiber being recorded for the first time (Gelfan, 1930b; Gelfan and Gerard, 1930). These findings, reminiscent of those seen in *Vorticella* stalks by Ida Hyde (1921) contrasted with earlier observations in muscle fibers that appeared to confirm the "All-or-None Law" (Pratt, 1917), as was accepted for nerve. Explanations proposed by Gelfan (Gelfan, 1930b, 1933) and Gelfan and Gerard (1930), for the graded contraction in muscle fibers, foreshadowed the eventual understanding of the role of the sarcomere.

In a footnote to their joint publication (Gelfan and Gerard, 1930), it is reported that a single muscle fiber impaled by a microelectrode "will always evoke a response as long as the fiber remains excitable…" [and that] "[w]ith the needle still inside, the fiber will eventually relax." It is suggested that the initial contraction is caused by injury to the membrane and that the relaxation is caused by healing of the membrane around the needle, as illustrated by a renewed contraction when the membrane is again injured as the needle is withdrawn. This sequence is reported to be repeatable up to 10 times if the fiber "is punctured by sharp and quick thrusts of a very fine needle." Gelfan and Gerard's "needles" (microelectrodes) were frog's Ringer-filled quartz capillaries hand pulled to have tip diameters of about 5 µm (Gelfan, 1927, 1930b).

With hindsight, because they were using their microelectrodes to stimulate single muscle fibers with extracellular currents, it is astonishing that they did

Figure 13. **Ralph Waldo Gerard (1952).** Image courtesy of the Special Collections Research Center, University of Chicago Library.

not, apparently, apply a stimulating current through an electrode while it was inside a relaxed fiber. Likewise, because Gelfan had already measured membrane potentials in *Nitella* using his microelectrodes (Brooks and Gelfan, 1928), why did he not also try this in the muscle fibers? Nevertheless, the extraordinary prescience of Gelfan's experiments seems to have been all but forgotten. Gelfan has turned out to be a sad figure in physiology, no longer recalled for his early brilliance (although he was awarded a Guggenheim Fellowship to work with Adrian in the United Kingdom in 1932) and later losing his position at Yale in the McCarthy era when his wife had been interrogated and accused of being a Communist (New York Times, 1952).

Gerard's interests had long been focused very firmly on the nervous system, and in 1930, this led him to invite to his home in Chicago "everyone working on nerve who was present at the meeting of the American Physiological Society, that year" (Gerard, 1975). This became the "Axonologist group" (Gerard, 1975; Magoun and Marshall, 2003), which at first included Philip Bard, George Holman Bishop, Hallowell Davis, Joseph Erlanger, Wallace Osgood Fenn, Alexander Forbes, Herbert Spencer Gasser, Ralph Stayner Lillie, Grayson Prevost McCouch, Francis Otto Schmitt and, later, many others (including Kenneth Stewart "Kacy" Cole).

This group will have known about the use of capillary and micropipette electrodes in monitoring transmembrane potentials in giant plant cells (Osterhout, 1931; Umrath, 1933) and about similarities between conduction of action currents in nerves and muscles and those in plant cells (Blinks et al., 1929; Umrath, 1929). After all, Bishop (Gelfan and Bishop, 1932, 1933) and Cole (Hogg et al., 1934) published work themselves using glass micropipette electrodes in experiments on skeletal muscle and embryonic rat cardiac myocytes, respectively.

Archibald Vivian Hill, with whom Gerard had worked in 1926–1927 in Cambridge, United Kingdom, quickly recognized Osterhout's contribution in his book, *Chemical Wave Transmission in Nerve* (Hill, 1932), and Alan Lloyd Hodgkin, also of Cambridge, later wrote that Osterhout was one of the people who had impressed him most (Hodgkin, 1977) and that he had read Osterhout's review (Osterhout, 1931) as an undergraduate (1932–1935). In contrast, in the mid-1930s, the American axonologists were already displaying some amount of hubris. Conscious that their field had been elevated "to a position of dominance in physiology" (Magoun and Marshall, 2003), they "almost strutted the corridors" (Marshall, 1987). According to Hodgkin (1977), they were also "thoroughly skeptical both of the membrane theory in general and of the local circuit theory [of nerve conduction] in particular." Both of these hypotheses had been espoused by the plant physiologists for action current propagation in the giant plant cells (Osterhout and Hill, 1930; Osterhout, 1931, 1934) in line with Ralph Stayner Lillie's proposals for the mechanism of impulse conduction in protoplasm and in inorganic models (for an extensive historical and topical review, see Lillie, 1922).

In this era, many microelectrode studies in large animal cells (typically represented by echinoderm eggs and *Amoeba*) served only to reinforce the view that, compared with plant cells, little or no potential difference existed across their plasma membranes (Taylor

and Whitaker, 1926; Gelfan, 1931; Buchthal and Péterfi, 1937; Rothschild, 1938). Measurements made in *Paramecium* seemed to be an exception (Kamada, 1934). It was not until much later (Tyler et al., 1956), when typical potentials of −10 to −60 mV were found, that an explanation for the earlier results from echinoderm eggs, consistent with failure to penetrate the plasma membrane, was proposed and accepted.

A number of unlucky attempts at impaling animal cells of more normal size, and especially at interpreting the results, occurred during this period. Precocious findings, suggesting an action potential overshoot that was not believable at the time (Cole, 1968), were obtained from embryonic rat cardiac myocytes in tissue culture using micropipette electrodes of around 2 μm in tip diameter (Hogg et al., 1934). It is surprising that the concept of the electrical action spike of nerve and muscle had been so fixed, as being a membrane potential decay to zero (Bernstein [1902], and see also Hodgkin and Huxley [1945]), that earlier strong indications of overshoot (Bernstein, 1868; Hermann, 1881; Burdon-Sanderson and Gotch, 1891) had been dismissed as anomalies (Grundfest, 1965). Frog skeletal muscle fibers, too, had been impaled with fine micropipette electrodes in efforts to measure potential differences at rest and during contraction (Buchthal and Péterfi, 1934). A sudden large potential change was indeed observed between one electrode in the Ringer solution bathing a muscle and another electrode as it was inserted nearby into the sarcoplasm of a single fiber. Because the initial potential difference decayed (probably because of leakage around the electrode) "in wenigen Sekunden" (in a few seconds) back to zero, however, it was interpreted as an injury potential and not as an indication of a normally existing potential difference across the sarcolemma. More emphasis was placed on the "Ruhepotentiale" (resting potential) between two electrodes situated at different distances apart on the surface of the sarcolemma or between two similarly separated electrodes inserted into the sarcoplasm. It is worth noting that the galvanometers and electrometers in use at the time were capable of recording these potentials with reasonable accuracy even using microelectrodes with micrometer-sized lumens and quite high resistance.

Utilization of the squid giant axon constituted the major advance in the study of animal cell membrane potentials. As with the original giant plant cell studies, the capillary electrode technique was applied, but now using longitudinally inserted intracellular glass cannulae of about 100 μm in diameter. In due course, the squid giant axon and amplifiers with sufficiently high input impedances and frequency responses, along with cathode ray oscilloscopes, allowed a complete analysis of the nerve action potential, its overshoot, and its propagation by Hodgkin and Andrew Fielding Huxley (Hodgkin and Huxley, 1939, 1945) and their colleagues and successors.

In the autobiographical memoir of his scientific life, Gerard tells that he "came in contact with nearly all workers on the nervous system, in laboratories or clinics" during a year in Europe in the mid-1930s (Gerard, 1975). If true, he must surely have gone to Graz, Austria, where Umrath was using glass microelectrodes of 3–7-μm diameter (Umrath, 1933) and had been publishing studies of electrical excitability in both nerves and giant algal cells (Härtel and Heran, 1986). Indeed, Ernst Florey (Florey, 1966) has written in his textbook, *An Introduction to General and Comparative Animal Physiology*, that, "After a visit to Umrath, Gerard brought the technique [of hand-pulling glass microelectrodes] to the United States."

Perhaps with this recent experience in mind, by the late 1930s, Gerard had developed a keen interest in the possibility of producing microelectrodes to use in individual neurons to monitor their electrical activity (Gerard, 1975; Kety, 1982). Anyway, in about 1940, he charged his PhD student, Judith Ethel Graham (Fig. 14), with the manufacture of glass micropipette electrodes, and these proved to be fine enough to make reasonably reliable measurements of membrane potentials in frog skeletal muscle fibers. Using these microelectrodes, albeit relatively large in tip diameter (5–10 μm) and filled with isotonic KCl, Graham succeeded in recording resting potentials of about −40 to −75 mV (average −54 mV) in the frog muscle fibers (Graham et al., 1942). Later, with sharper electrodes (<5 μm), more substantial membrane potentials to −80 mV (average −62 mV) were obtained (Graham and Gerard, 1946). Currents were also passed through these micropipettes to stimulate action potentials and contractions and to determine excitatory strength duration curves (Gerard and Graham, 1942; Graham and Gerard, 1946). The microelectrodes used by Graham and Gerard, although of glass rather than quartz, were similar in construction and tip diameter to those produced by Gelfan in 1927 and used by Gelfan and Gerard in 1930, as outlined earlier.

Graham had married a fellow student at the University of Chicago, Ithiel de Sola Pool, in 1938 and graduated with a BSc in 1939. She then began graduate studies and worked as an assistant in Gerard's Physiology Department. While still a graduate student, Graham and her husband moved to Geneva, New York, where she taught physics at Hobart and William Smith Colleges and gave birth to two sons. Despite these serious interruptions to her program, she completed her PhD in 1946, although after this she did no more research using microelectrodes. In 1953, the family moved to California, where she undertook hematological research and, in 1972, became a full professor at Stanford University. She achieved international renown, as Ju-

Figure 14. **Judith Ethel Graham (Pool) (1943).** Image courtesy of Jeremy Pool. Jeremy Pool is a son of Judith Ethel Graham Pool.

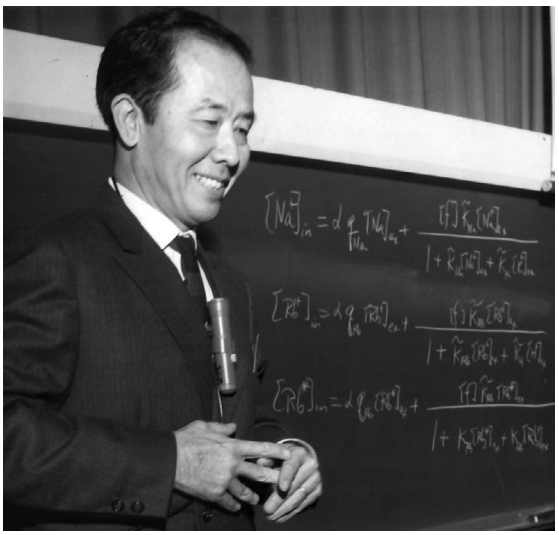

Figure 15. **Gilbert Ning Ling.** Image courtesy of the Ling Family Collection.

dith Graham Pool, in 1964, for the discovery of a simple and inexpensive way to separate anti-hemophilic Factor VIII from blood plasma (Pool et al., 1964). Her method enabled the harvesting and storage of Factor VIII while retaining the remaining blood plasma sterile and available for other uses. This contribution to blood banking saved the lives of thousands of hemophiliacs.

Glass micropipette electrodes, superior to those of Graham, were manufactured by Gilbert Ning Ling (Fig. 15) soon after his arrival from China, in 1946, to work with Gerard (see, e.g., Graham and Gerard [1946], where Ling's microelectrodes are first mentioned). Ling recognized the limitations of Graham's microelectrodes and set about perfecting the art of hand pulling the micropipettes, which had not advanced (and even seemed to have regressed) since the original examples prepared by Barber, Kite, Taylor, Gelfan, and others. Ling's particular contribution was to establish a source of stable heat. All previous workers had pulled their pipettes over small gas burners—down to 1 mm in diameter. As Ling realized, these were too unstable in drafts of any kind, leading to great inconsistencies in electrode characteristics. Ling pulled his electrodes after heating his glass tubing in the upper edge of a tall (10–12 cm), large-diameter (1 cm) flame of an air-gas blow torch with the air supplied by a reliable air pump (Ling, personal communication). It is noteworthy that Barber himself, although he advocated the use of a microburner with the lowest possible flame, also recommended that the preparation area "be free from drafts of air" (Barber, 1914), and Péterfi used a curved shield close to his microburner to protect against air currents (Péterfi, 1923). The result was that Ling could consistently obtain pipettes of <0.5 µm in tip diameter (Ling and Gerard, 1949), a skill once again matching that of Kite (1912). Using these pipettes, filled with isotonic KCl by boiling under intermittent pressure, Ling and Gerard published membrane potentials averaging −78 mV in vitro (−85 mV in vivo; Ling and Gerard, 1949). For a lengthy period afterward, similar glass micropipette electrodes were known as "Ling–Gerard" electrodes, although this eponym has largely fallen into disuse since the advent of patch-clamp electrodes and a decrease in the use of sharp glass micropipette electrodes.

Like Judith Graham, Ling soon abandoned the use of microelectrodes, turning his research attention toward largely theoretical considerations of his "Association-Induction Hypothesis" (Ling, 1962) and his Polarized-Oriented Multilayer theory of cell water (Ling, 1965). These argue against the existence of membrane ion pumps and diffusion potentials, but neither one has gained acceptance in mainstream biophysics/biochemistry (although Ling has achieved a considerable personal reputation in his self-promotion of them). Although he has made some contributions to protein chemistry, his more extreme proposals have been at least partly responsible for stimulating conventional research that has resulted in general acceptance of membrane theory, protein channels and transporters, and the molecular biology and genetics associated with them.

According to his scientific autobiography, Gerard was nominated for the Nobel Prize "for developing the microelectrode" (Gerard, 1975). He went on to write what amounts to a disclaimer: "and this seems to be my best-known contribution, although I personally have been more excited about some discoveries and interpretations than about methodological contributions." As Gerard is implying, he did, in fact, have other more significant and worthy input to Physiology, Psychology, Psychiatry, and Philosophy (cf. Kety, 1982).

Nevertheless, there does seem to be some attempt at justifying his Nobel nomination in his autobiography, where he attributes the idea of using glass micropipettes

to a personal experiment he initiated as an undergraduate at the University of Chicago while taking a course in histology. He recounts (Gerard, 1975; cf. Kety, 1982): "there was still great argument among histologists as to how much of what one saw under the microscope was present in the living cell or tissue and how much was fixation artefact. (This even included myofibrils.) I suggested to Professor Bartelmez that if a quartz needle was moved steadily across a living muscle fiber, the tip would move smoothly if the protoplasm was homogeneous but in a sort of cogwheel fashion if viscous fibrils were imbedded in fluid sarcoplasm, and this could be followed by reflecting a beam of light from a mirror attached to the needle. He was enthusiastic, and unearthed from a storage shelf and presented to me the original micromanipulator that had been developed in the department by Kite. Protoplasm proved to be vastly more viscous than I had dreamed, and this particular experiment did not work."

It seems unlikely that this can have been Gerard's real inspiration for utilizing glass micropipettes. As already mentioned, there appear to have been many occasions between 1920 and 1940 in which he must have observed their method of manufacture, have noted that they could impale muscle and plant cells without lasting damage, and have been aware of their use in measuring transmembrane potentials. Almost nothing of these precedents is cited by Gerard in his microelectrode publications, the substantial contributions of the plant physiologists being especially notable by their absence.

Gerard's own early work with Gelfan, in which they stimulated single muscle fibers (Gelfan and Gerard, 1930), is cited only in passing by Graham and Gerard (1946) (and not at all by Ling and Gerard [1949]) and then without mentioning that sharp glass micropipettes had been used as the stimulating electrodes in that earlier work or that muscle fibers had been impaled.

If Gerard had learned how Umrath hand pulled micropipettes (Florey, 1966), as mentioned above, it is unacknowledged by Gerard. Apparently, also, no mention of any prior history of this kind is made in the theses of Graham and Ling. This lack of citation has been used by Stuart and Brownstone (2011) to argue against any influence of Umrath on the development of the microelectrode. In contrast, Florey had been a student of Umrath at the time of the Ling and Gerard (1949) publication, gaining his PhD from Graz in 1950 (Krnjević, 2010). Because there is no obvious reason why Florey would have concocted his account of Gerard's earlier introduction to Umrath's method of preparing micropipette electrodes, we might presume that Umrath had informed Florey of it.

Gerard was also less than generous regarding the input of Graham and Ling to the work for which he received his Nobel nomination. His autobiography scarcely acknowledges these co-contributors, who seem to have personally performed the experiments, mentioning each of them just once (Gerard, 1975): "With Judith Graham, I *developed* [my italics] a salt-filled capillary with a tip small enough (up to five microns) that a muscle fiber could be impaled without excessive damage. Gilbert Ling soon picked up these studies, and the electrode was pushed down to a few tenths of a micron…."

Indeed, it was Ling who completed the penultimate step in the generation of the modern glass micropipette electrode, being the first to produce and use examples with tips that were consistently fine enough to avoid significant damage when impaling normal-sized animal cells (Ling and Gerard, 1949; and see also Graham and Gerard, 1946). Finally, however, in a renaissance of Taylor and Whitaker (1927) technology, William Leo Nastuk and Hodgkin had by December 1948 (Hodgkin and Nastuk, 1949; Nastuk and Hodgkin, 1950) filled micropipettes, still pulled by hand, with 3 M KCl, thereby reducing pipette resistance, minimizing any junction potential between pipette and cytoplasm, and obtaining resting potentials in frog muscle close to −90 mV.

From the first years of the 1950s, glass micropipettes for use as microelectrodes began to be pulled, no longer by hand, but by mechano-electrical pullers (e.g., Alexander and Nastuk, 1953) that became ever more sophisticated (see, e.g., Brown and Flaming, 1986). Their use has largely eliminated inconsistencies and allowed custom designs for length, taper, and tip diameter and for characteristics dependent on these when filled with an appropriate electrolyte solution.

Glass micropipette electrodes have since been used to stimulate and record from a wide variety of plant and animal cells and tissues. They have been the major instrument by which the electrical characteristics and electrical activity have been determined in everything from single-celled organisms to interacting deep brain neurons during defined complex behavior.

This historical account shows that progress in developing and using the glass micropipette electrode was haphazard with numerous inventions and reinventions, with advances and regressions, with missed opportunities and false starts, and with both mistaken and correct interpretations of results. Of course, few paths toward other technical achievements or discoveries have been straightforward or direct, and this one, resulting finally in the widespread adoption of the 3 M KCl–filled glass micropipette electrode, was no exception.

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