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Historical Background

The quest for a scientific understanding of electrical signaling in the nervous system began more than a century and a half before the 1952 papers of Hodgkin and Huxley. In 1791, Luigi Galvani (1737–1798) reported his discovery of 'animal electricity'—the electrical processes somehow generated by biological tissue to transmit signals from nerves to muscles. Like many scientific discoveries, the initial observation was incidental: while a metal scalpel was in contact with a nerve in the leg of a decapitated frog, the muscle contracted whenever a nearby frictional machine—a device that generated static electricity by rubbing two materials like glass and wool together—emitted a spark. Apparently, it was one of Galvani's assistants who first noticed the coincidence of the two events. As Galvani reported,

> He, wondering at the novelty of the phenomenon, immediately apprised me of the same, wrapped in thought though I was and pondering something entirely different. Hereupon I was fired with incredible zeal and desire of having the same experience, and of bringing to light whatever might be concealed in the phenomenon. Therefore I myself also applied the point of a scalpel to one or other crural nerve at a time when one or other of those who were present elicited a spark. The phenomenon always occurred in the same manner: violent contraction in individual muscles of the limbs, just as if the prepared animal had been seized with tetanus, were induced at the same moment of time in which sparks were discharged. (Galvani 1791, trans. Green 1953 p. 24)

Galvani and his assistants eventually determined that this striking phenomenon was reproducible, and, subsequently, that simply touching the metal contacting the nerve with a different kind of metal was sufficient to induce contraction. From these observations, Galvani developed a theory, building on ideas of the Ancient Greeks, of an electric fluid inherent to the nerves, whose role was to generate muscle contraction. To defend his proposal against its most vocal opponent, Alessandro Volta (1745–1827), who asserted that electricity originated exclusively from dissimilar metals in contact and never from living organisms, Galvani conducted an experiment referred to as 'contraction without metals' (*contrazione senza metallo*). He reported that contraction could be induced simply by bringing the end of the nerve into contact with a nearby (damaged) muscle, which apparently depolarized the nerve sufficiently for it to fire (Galvani 1791, trans. Green 1953; Mauro 1969; NONC p. 163–173).[1]

Investigations of a possible electrical component to nerve signaling ('activity') and muscle contraction continued throughout the nineteenth century, although the controversy over the existence of animal electricity persisted for decades. These studies drew on electrical principles being discovered at the time—notably the relation among voltage, current, and resistance, articulated by Georg Ohm (1789–1854) in 1827—as well as new technologies in the form of measurement devices. After the development of the galvanometer as a tool to detect current, Leopoldo Nobili (1784–1835) and, later, Carlo Matteucci (1811–1868) demonstrated that current flow could be detected between two electrodes placed on an injured and an intact region of muscle—essentially the conditions of Galvani's contraction without metals; these results offered the first evidence of a voltage difference between the interior and exterior of the cell, or 'resting potential.' Remarkably, Matteucci later recanted, after difficulty reproducing his results in nerves. The line of research was continued by Emil Dubois-Reymond (1818–1896), who ultimately

[1] In addition to original articles, this section repeatedly cites four books, which are referred to by their initials:
NONC: *Nineteenth Century Origins of Neuroscientific Concepts.* E. Clarke and L. S. Jacyna (1987)
MII: *Membranes, Ions, and Impulse.* K. S. Cole (1968)
CDD: *Chance and Design: Reminiscences of Science in Peace and War.* A. Hodgkin (1992)
HNA: *The History of Neuroscience in Autobiography. Vol. 4.* A. F. Huxley (2004)
Full citations are given in the references.

recognized the unit of electrical signaling as a transient reduction in current flow between a region of intact nerve and its cut end, which he named 'the negative variation' (*der negative Schwankung*). Stated in modern terms, because a healthy region of resting nerve membrane has a substantial transmembrane potential (of about −60 mV) and a damaged region has a potential near zero, a voltage difference exists across these two sites, making it possible to measure current flowing between them. During an action potential, the transmembrane voltage of the intact region approaches zero, reducing the voltage difference. Consequently, the measured current changes from a high to a low value during electrical activity. The negative variation was thus the signature of the action potential (Schuetze 1983; NONC pp. 189–190, 196–211).

A student of Dubois-Reymond, Julius Bernstein (1839–1917), improved the measurement technique and reconstructed the time course and conduction velocity of the negative variation in nerve bundles (Bernstein 1868). Later, drawing on the work of Walther Nernst (1864–1941), Bernstein proposed the 'membrane theory,' arguably becoming the father of membrane biophysics. The theory stated that cells consisted of electrolytes encapsulated by a membrane that was relatively impermeable to all ions except potassium, which was found to be permeant through ion substitution experiments. Bernstein deduced that an electrical potential would exist across such a membrane and further proposed that the permeability of the postulated membrane would break down during electrical activity or 'irritability' (*der Reizung*). The resulting redistribution of ions would produce 'action currents' (*Actionsströme*), accounting for the negative variation (Bernstein 1902; MII pp. 6–9).

Evidence in favor of the membrane theory came some years later from Rudolf Höber (1873–1953), who was working under the guidance of Nernst (Höber 1910, 1912). Höber succeeded in measuring resistances of preparations of red blood cells by applying alternating currents to them under different conditions. He found that intact cells indeed had high resistances, but only when a low-frequency current was applied. The frequency sensitivity of the intact cell led to the concept of the membrane as a capacitor, a circuit element that filters low- but not high-frequency currents. In contrast, after hemolysis, the resistance of the red blood cell preparation was measured to be low regardless of frequency, indicating that the cell interior was conductive. These results provided evidence that the protoplasm was indeed composed of electrolytes. Bernstein's son reported that his father was highly gratified by Höber's experimental support of his theory. (MII pp. 6–7)

The biophysical characteristics of cell membranes, including the value of the capacitance and the conditions that generated the selective permeability (which was lost in dead cells), were studied further in a variety of cells, including guinea pig muscle and liver cells (Philippson 1921), kelp (Osterhout 1922), blood cell suspensions (Fricke 1925), and algae (Blinks 1928). By applying alternating currents to cell preparations and observing the phase shifts and frequency-dependent resistances, the results from different cells converged on the conclusion that most cell membranes had a specific membrane capacitance close to 1 $\mu F/cm^2$ and a thickness of ~33 Å (Fricke 1925). (MII p. 8)

The demonstration that living phenomena, including bioelectricity, could be described by physical laws motivated the drive to identify an electrical circuit whose properties would mimic a living cell. According to Cole (1968), perhaps the first such equivalent circuit (Philippson 1921) included a parallel resistance and capacitance, representing the membrane, in series with another resistance, representing the highly conductive cell interior (MII p. 9). The stage was set for the heroic age of membrane biophysics.

According to his memoir *Chance and Design*, Alan Lloyd Hodgkin (1914–1998) became interested in the field of physiology as an undergraduate at Trinity College at Cambridge University. Hodgkin's boyhood had been shaped by ornithological, botanical, and other natural pursuits, and physiology ran neck-and-neck with zoology as his specialization of choice for the research phase of his studies; botany, too, was a subject he apparently relinquished with some regret. Hodgkin began his independent research in physiology in 1934 (at age 20) on the question of whether the membrane of excitable cells underwent an increase in conductivity in association with the electrical signaling.

His father George, who had died in 1918, had also studied Natural Sciences at Trinity, and Hodgkin cites the studies of his father's friend and classmate Keith Lucas as particularly influential in his (Alan's)

choice of research direction (C&D p. 63). Between 1904 and his premature death in 1916, Lucas published 21 papers in the *Journal of Physiology* on the excitability of muscle and nerve, including the all-or-none nature of the contraction of skeletal muscle fibers, the refractory period, and a theory of excitation (Lucas 1909a, 1909b, 1910). Lucas was not only prolific but also precise about defining terms, in a style that seems to be echoed in Hodgkin's later scientific writing. In his 1910 paper, Lucas wrote:

> The word excitation has by somewhat loose usage become applicable to all or any of the successive processes which constitute the connecting links between the application of a stimulus to a nerve or muscle and the appropriate final response. The application of the stimulus is not infrequently spoken of as excitation. The immediate local effect of the stimulus is called by the same name. The disturbance which is conducted away from the seat of application of the stimulus is often called the wave of excitation. A muscle is even said to be excited when it contracts in consequence of a stimulus applied to its motor nerve. It seems therefore that we are bound to define precisely at the outset what is meant in this place by a theory of electric excitation.
>
> When an electric current is passed through part of a muscle fibre or nerve fibre there must be produced in the fibre a local physical alteration which is the immediate consequence of the current. This physical alteration provides the necessary condition for starting a disturbance which is then propagated away from the seat of application of the current. A theory of electric excitation means, as here used, a theory of the physical nature of that local alteration within the fibre which constitutes the necessary condition for starting the propagated disturbance. It is not a theory of the nature of the propagated disturbance, though no doubt it may ultimately lead to such a theory. Still less is it a theory of the more remote disturbance which constitutes contraction. (Lucas 1910)

More contemporary scientific influences on Hodgkin's choice of research topic were the early studies of cell capacitance by the American botanist Winthrop van Osterhout (1871–1964) and his student and collaborator Lawrence R. Blinks (1900–1989), who studied *Nitella*. Cells from this freshwater alga produce action potentials of a few seconds in duration, later found to depend on efflux of chloride followed by potassium (Gaffey & Mullins 1958). Hodgkin particularly recalls being influenced by the study of Blinks (1930), which stated:

> It is an interesting property of the cells of *Nitella* to be stimulated by electric current, and to transmit that stimulus as a negative variation, giving a typical diphasic action current comparable to that observed in muscle and nerve. (Blinks 1930)

Using a Wheatstone bridge (see Appendix 3.4), Blinks measured the resting resistance across the long axis of cylindrical *Nitella* cells, as well as the change in resistance upon stimulation. Blinks concluded his paper with the following description relating current (the negative variation), conductance (the resistance change), and voltage (potential difference) during activity:

> The study of these phenomena is not complete, but the outstanding effects on resistance may be indicated. The crest of the negative variation is really a depression of the P.D. [potential difference] at the contact nearly to zero. At the same time the resistance across the protoplasm is likewise greatly lowered and may fall momentarily to 0.1 megohm [from >3 megohms], about as in contact with 0.1 M KCl. This suggests that the cathodic stimulation may consist in the movement of sufficient K^+ ions in an outward current (from the sap to the external solution) to reach approximately such a concentration just outside the protoplasm). (Blinks 1930)

Hodgkin states that Blinks' study motivated him to begin the research phase of his studies by investigating conductance changes in nerve (C&D p. 63). Working with frog myelinated sciatic nerve, in which a short segment was cooled sufficiently to block firing, he found that stimulating an action potential on one side of the block could increase the excitability of the nerve beyond the block. In the first of a pair of papers, Hodgkin comments on his own study with a succinct encapsulation of the scientific method:

> The experiments described in this section do not throw much light upon the mechanism of summation, but they must precede any detailed analysis of the process. (Hodgkin 1937a)

In other words, observation is the necessary precursor to hypothesis. Hodgkin's second paper provides the detailed analysis. Complementing his observations with a series of calculations based on cable theory, he provided evidence that excitability must spread as a result of current flowing in local circuits: down the axons, out the membranes, backward along the outside of the axon, and back in across the membrane. By this electrotonic mechanism, a local change in voltage could be transmitted to more distant regions (Hodgkin 1937b).

In an amusing side note that contrasts vividly with twenty-first century scientific training, Hodgkin's memoir recalls the response of Joseph Barcroft, the head of the laboratory in which he worked, to Hodgkin's query about whether his manuscript required approval before submission to a journal:

> He was quite taken aback and explained first that we did not do anything like that in Cambridge and, second, that anything I wrote was entirely my own affair. (C&D p. 68)

Hodgkin's published papers drew the attention as well as the skepticism of the American physiologists Herbert Gasser (1888–1963) and Joseph Erlanger (1874–1965), who shared the Nobel Prize in Physiology or Medicine in 1944 for their collaborative work on conduction velocity in axons. Erlanger expressed polite scientific doubt about Hodgkin's results, based largely on the absence of detectable ephaptic stimulation of neighboring axons in his own experiments; Gasser invited Hodgkin to spend a year in his lab at the Rockefeller Institute. Accordingly, Hodgkin went to New York. He spent the following summer (1938) at the Marine Biological Laboratory at Woods Hole, on Cape Cod in Massachusetts, where he met and worked with Kenneth Cole (C&D pp. 74–78).

Like Hodgkin, Kenneth S. (Kacy) Cole (1900–1984) was influenced by Blinks and vice versa; indeed, Cole is thanked for making measurements reported in Blinks (1928). Cole completed a PhD in physics in 1926 and gained experience working on the electrical properties of cell membranes with Hugo Fricke (1892–1972) during the summer of 1923. Ultimately, Cole became interested in the relationship between 'irritability,' electronics, and oscillations. His first published work on nerve, a note to *Science* in 1934 (Cole 1934), started with a statement on the scientific power of Fourier analysis and went on to analyze the system in terms of resistance, capacitance, and inductance. His experimental observations of frog nerve responses to stimulation deviated from the expected linear behavior of these elements. He therefore proposed a modified equivalent circuit for the membrane, which included a capacitance and a hypothetical resistance that, like a capacitor, would change its impedance as a function of the frequency of applied current. In this scenario, a conductance change in response to high-frequency alternating current would lead to the damped oscillation that is the action potential. The fundamental implication, which followed from the nature of a capacitor, was that *current* was the underlying independent variable that drove the conductance change.

Measurement of the conductance changes, and the stimuli that drive them, therefore became paramount. Cole, with collaborator Howard Curtis (1906–1972), repeated Blinks' 1930 studies in *Nitella* cells with an interest in testing whether alternating current of different frequencies affected the conductance. Recognizing that Blinks' longitudinal measurements across the long, cylindrical cells might be dominated by protoplasmic rather than transmembrane impedance, Cole and Curtis connected a Wheatstone bridge in the perpendicular orientation and made recordings of the transverse resistance across the ~0.45 mm diameter of the cell. They concluded that the cell membrane had negligible conductance at rest (Curtis & Cole 1937). Upon shock-stimulation of an action potential that propagated slowly (1 cm/sec) along the length of the cell, however, the impedance of *Nitella* decreased, such that the conductance peaked on the rising phase of the extracellularly recorded action potential. Cole and Curtis emphasized the distinction between the extracellularly recorded action potential and the actual transmembrane voltage, but they suggested that such a voltage must exist that would be 'intimately related' to the transmembrane conductance. They illustrated another modified equivalent circuit for a membrane that included a capacitor in parallel with a serial combination of a battery and resistor (Cole & Curtis 1938).

Comparable transverse recordings to investigate the basis of excitability of animal cells promised to be informative, but nerve fibers on the physical scale of *Nitella* cells were unknown. In 1936, however,

John Zachary Young (1907–1997) described the squid (*Loligo forbesi*) nervous system as having giant fibers arising from giant cells and forming giant synapses (Young 1936). Cole and Curtis recognized the squid giant axon, which was about 0.6 mm diameter, as a potentially useful preparation for the study of nerve impulses (Curtis & Cole 1938). Working with these axons, they made recordings of the capacitance and conductance across the axon during rest and activity, comparable to those they had made in *Nitella*. In what is arguably the most famous figure from their work together, they illustrated the conductance changes that occurred during a propagating action potential (Cole & Curtis 1939, reproduced in the final paper of the Hodgkin-Huxley 1952 series). Despite the conductance change, the capacitance remained constant throughout the action potential, indicating that the membrane itself did not break down during activity. The mechanism of the conductance change, however, remained a mystery. In their discussion, Cole and Curtis commented, somewhat prophetically,

> In contrast to the *Nitella* results, it will be noticed that for the squid axon the recovery of the action potential is completed considerably before that of the membrane resistance, but it seems likely that when this difference can be explained the whole phenomenon of excitation and conduction will be fairly well understood. (Cole & Curtis 1939)

Interestingly, Hodgkin apparently walked in on Cole and Curtis's classic experiment. In his memoir, he recalls

> arriving in Cole and Curtis's room there and seeing the increase in membrane conductance displayed in a striking way on the cathode ray tube. (C&D p.115)

At Rockefeller, Hodgkin had been working on single axon fibers from the crab, a preparation he had started on back in Cambridge; at Woods Hole, he was introduced to the preparation of the squid giant axon by Cole. His memoir quotes a letter to his mother from mid-June 1938:

> As you know, I spend my time working with single nerve fibre from crabs, which are only about 1/1000 of an inch thick. Well, the squid has one fibre which is about 50 times larger than mine and Cole has been using this and getting results which make every one else's look silly. Their results are almost too exciting because it is a little disturbing to see the answers to experiments that you have planned to do coming out so beautifully in someone else's hands. No, I don't really mean this at all, what I do dislike is the fact that at present English laboratories can't catch squids so that I don't see any prospect of being able to do this myself. (C&D p. 119)

While at Woods Hole, Hodgkin used both squid and crab to conduct a straightforward test of the local circuit hypothesis. If the current indeed flowed in loops, he reasoned, then the speed of propagation of the action potential would be influenced by the magnitude of the resistance *extracellular* to the axon. This idea gave rise to specific testable predictions: increasing the external resistance should impede current flow in the circuit, slowing the conduction velocity; reducing the external resistance should facilitate current flow, speeding conduction. The predictions were fulfilled. The results provided strong evidence that voltage indeed spreads along the axon as a consequence of local circuit currents, carried by ions, flowing through the resistances within the axon, out across the membrane, extracellularly along the axon, and back across the membrane into the axon (Hodgkin 1939).

In Hodgkin's final weeks at Woods Hole that summer, he and Cole worked together on a fundamental problem that interested them both: measuring the resting resistance of the squid axonal membrane (Cole & Hodgkin 1939). Hodgkin's memoir quotes part of a letter to his mother, 'This is the first time that I ever collaborated with any one, and I never realized till now how much nicer it is than working alone.' (C&D p.117) Upon returning to Trinity College in Cambridge, Hodgkin began what were intended to have been three years of pure research. That autumn (1938), he supervised a laboratory practical class for physiology students, which included the 20-year-old Andrew Huxley.

Unlike Hodgkin, who confessed 'I have always been rotten at making things' (C&D p. 71), the propensities and enthusiasms of Andrew Fielding Huxley (1917–2012) were mechanical. Huxley begins his memoir with a quote by his grandfather Thomas Huxley, the famous proponent of Darwin's theory of evolution, followed by a comment on its pertinence to himself:

> T.H. Huxley wrote a short autobiography which includes the following passage:
>
> 'As I grew older, my great desire was to be a mechanical engineer, but the Fates were against this; and, while very young, I commenced the study of Medicine under a medical brother-in-law. But, though the Institute of Mechanical Engineers would certainly not own me, I am not sure that I have not, all along, been a sort of mechanical engineer *in partibus infidelium*. . . . The only part of my professional course which really and deeply interested me was Physiology, which is the mechanical engineering of living machines.'
>
> Much of the same could be said of me: my boyhood interests were mainly mechanical, and I entered Cambridge University with the intention of specializing in physics and becoming an engineer. My subsequent interest in physiology is exactly described by the phrase 'the mechanical engineering of living machines,' and a substantial part of my work has been the design and construction of instruments needed for my research. (HNA p. 284)

Huxley describes a childhood of working with Meccano, microscopes, and a lathe that he kept and used all his life. During his first two undergraduate years at Cambridge, he studied physics, chemistry, and mathematics. On the advice of a senior classmate, he chose physiology as an elective science course. Huxley writes,

> [Ben Delisle Burns] told me that physiology was a lively subject in which even in the first year newly discovered things, and things still controversial, were taught, unlike the situation in physics or chemistry. (HNA p. 290)

Huxley went on to specialize in physiology, and among the courses he took at the beginning of the research phase of his training was the laboratory practical taught by the young Hodgkin.

Meanwhile, having purchased new equipment with an unexpectedly large grant of £300 from the Rockefeller Institute, Hodgkin returned to recording from crab axons (C&D p. 124). He describes an early experiment, intended primarily to test out his DC amplifier, in which he estimated the magnitude of the action potential relative to that of the resting potential. These voltages had to be measured between the outside of the axon and a region where the membrane potential had likely been brought to zero, either by injury, as in the days of Dubois-Reymond, or by increasing external potassium ions to match the intracellular concentration; with this method, only relative rather than absolute magnitudes could be compared. Bernstein's membrane theory predicted that the action potential would bring the membrane potential from a resting negative value (now known to be near −60 mV) to a value close to zero (e.g., −10 mV). Thus, the magnitude of the action potential (in this example, 50 mV) might be close or even equal to, but never greater than, the magnitude of the resting potential (in this example, 60 mV).

Hodgkin's results, however, suggested something quite different: the action potential appeared to have a *greater* magnitude than the resting potential. With his student Huxley joining him for some of the experiments, Hodgkin repeated the experiments not only in crab but lobster (C&D pp. 130–131). During the following summer (1939), he went to the Laboratory of the Marine Biological Station at Plymouth, in Devon, England, where squid could be caught for experiments. Huxley joined him, having turned down a research offer that would have let him pursue his interest in microscopy, and began his research at Plymouth with some unpromising experiments on the viscosity of squid axoplasm (HNA pp. 291–292). Hodgkin recalls,

> Huxley said that he thought it would be fairly easy to stick a capillary down the axon and record potential differences across the surface membrane. (C&D p. 133)

Huxley's memoir, however, credits Hodgkin for the idea:

> Hodgkin suggested pushing an electrode down inside so as to record the membrane potential directly between axoplasm and external fluid. (HNA p. 292)

The method worked, and the results were dramatic. In Huxley's words,

> We immediately found that the amplitude of the action potential was much greater than the resting potential, so that the internal potential went considerably positive at the peak of the action potential. This was contrary to the then current belief, although Hodgkin already had hints of an 'overshoot' from external recordings on single fibers from crabs and lobsters, although this was not published until later. (HNA p. 292)

Hodgkin continues,

> Andrew Huxley and I were tremendously excited about the potentialities of the technique and started other tests. . . . However, within three weeks of our first successful impalement, Hitler marched into Poland and I had to leave the technique for eight years until it was possible to return to Plymouth in 1947. (C&D p. 133)

Thus, after making these extraordinary observations in August 1939, Hodgkin and Huxley were forced to suspend their research, owing to England's entry into the war in Europe. Both entered military service shortly thereafter—Hodgkin worked on radar and Huxley worked on gunnery—but they published an initial report of their findings in October (Hodgkin & Huxley 1939), in what Hodgkin termed 'a cautious note to *Nature*' (C&D p. 135). They reported that the total amplitude of the action potential was about 90 mV. The resting potential was near −50 mV; thus, the action potential overshot 0 mV, reaching about +40 mV at its peak. Hodgkin and Huxley did not emphasize this result, however. The technique was novel, and they pointed out that while the 90-mV total amplitude was likely reliable, the absolute voltage values might be skewed by liquid junction potentials. Nevertheless, both scientists were fully aware that the switch in polarity of the membrane potential was too substantial to be attributable to measurement error and that it constituted evidence against the hypothesis that the electrical signal was a complete breakdown in the selective permeability of the membrane. Instead, it suggested an alternative, active process driving the voltage to positive values, which they would not be able to explore experimentally until after World War II.

Meanwhile, in America, Cole continued his studies of the squid axon. In the summer of 1938, at the end of his collaboration with Hodgkin, Cole had observed what looked like oscillations following the action potential (C&D p.117). Oscillations are reminiscent of resonance, and resonance in electrical circuits can be generated by an inductor and capacitor in parallel (see Appendix 2.6). Cole, who had made meticulous measurements of capacitance, was still seeking the correct equivalent circuit for an excitable membrane, and the oscillations made the idea of an inductor-like element in the membrane seem plausible. In a series of papers, Cole explored this idea, adding an inductor into his evolving equivalent circuit of the membrane (Cole & Baker 1941; Cole 1941). Cole and Curtis also figured out how to insert an electrode into the squid axon to measure transmembrane potential. Unlike Hodgkin and Huxley, who had used a silver wire coated with silver chloride, Cole and Curtis used a 'needle' electrode, a glass micropipette filled with a potassium chloride solution isotonic with seawater. The capacitance of the glass introduced a lag, for which Cole and Curtis compensated electronically. Even slight overcompensation of such circuits, however, can lead to oscillations ('ringing'); in this case, the ringing overlaid the oscillation-like voltage swings of the action potential, distorting its waveform and exaggerating its magnitude. In 1942, Cole and Curtis therefore reported (incorrectly) that the absolute voltage of the action potential, measured from resting potential to peak, could be as much as 150 mV. They recognized the significance of the overshoot in refuting the original formulation of Bernstein's hypotheses, but its explanation eluded them:

> Thus during the passage of an impulse the membrane potential is momentarily reversed in sign, so that the outside may be as much as 110 millivolts negative with respect to the inside. This fact throws doubt on the simple explanation of the action potential as a passive depolarization of the membrane or abolition of the resting potential. With the further observations of wide variability in the size of the

> action potential with little if any change of the resting potential, it is reasonable to suppose that a separate mechanism is responsible for the production of each. Thus the resting potential may be an electrical measure of the energy made available by metabolism and the action potential an index of the ability of the membrane to utilize this energy for propagation. (Curtis & Cole 1942)

Regarding mechanism, Cole and Curtis did experiments designed to test whether the potentials that they measured were sensitive to ions in the bathing solution, as might be expected for a conductance-based phenomenon. Indeed, the idea was already afoot that sodium ions might be responsible for the depolarizing phase of the action potential, a possibility that came to be known as 'the sodium hypothesis.' Cole and Curtis failed, however, to detect much responsiveness of the action potential to the loss of external ions, including sodium:

> Removing all ions by circulating isosmotic dextrose increased the potential only slightly (3 to 5 millivolts) higher than it was raised by removal of potassium alone. Likewise, the height of the action potential was not appreciably affected by these procedures. (Curtis & Cole 1942)

Instead, they raised the possibility of an inductance-based resonance:

> However, there may be an explanation of this phenomenon on the basis of a passive depolarization. A membrane inductance has been observed, (Cole and Baker, '41) in this fiber of 0.2 henries per cm.^{2} and this, in conjunction with the membrane capacity of 1 microfarad per cm.^{2} (Curtis and Cole, '38) forms a resonant circuit. It has been possible to explain several phenomena of peripheral nerve on the basis of an equivalent membrane circuit involving capacity, resistance, and inductance (Cole, '41). The explanation of the present phenomenon in terms of this equivalent circuit is not available, but it seems possible that a complete solution of the problem on the basis of the cable equations may yield an adequate explanation. (Curtis & Cole 1942)

These observations, by distinguished and reputable scientists, of action potentials with peaks far surpassing the predicted sodium equilibrium potential and waveforms insensitive to changes in sodium concentration, made it seem highly unlikely that nerve activity resulted from an increase in membrane permeability to sodium ions. The insensitivity of the action potential to external sodium ions was also propounded by Rafael Lorente de Nó (1902–1990), a member of Gasser's department at the Rockefeller Institute who conducted extensive studies on the question. The reason for these erroneous observations was that the perineurium that ensheaths axonal fibers (within the epineurium that surrounds nerves) contains a layer of epithelial cells that form a diffusion barrier to ions. The ion-exchange experiments were therefore flawed. Even after evidence for an ion impermeable membrane began to accumulate, resistance remained strong. As late as 1950, in a paper rather boldly titled, *The ineffectiveness of the connective tissue sheath of nerve as a diffusion barrier*, Lorente de Nó wrote:

> The concept that the connective tissue sheath, or rather the epineurium, of frog nerve is an effective diffusion barrier was introduced by Peng and Gerard ('30). The concept was dismissed by Lorente de Nó, who, from his observations on the action of a number of substances upon frog nerve, concluded that 'it is utterly impossible to believe that the connective tissue sheath of frog or bullfrog nerve could act as a diffusion barrier that would delay for considerable periods of time the penetration of solutes into the nerve (Feng and Gerard, '30),' and that 'the connective tissue sheath is freely permeable to solutes, be they ionized or not' (Lorente de Nó, '47a, vol. 1, p. 23). Recently, however, the concept that the epineurium of frog nerve is an effective diffusion barrier has been reintroduced in the literature by several authors. (Lorente de Nó 1950)

He recognized the legitimate problem this novel concept posed for the scientific literature, to which he had been no small contributor:

> It must be realized that the statements made by Feng and Liu, Hodgkin, Huxley and Rashbass and Rushton have created an exceedingly serious situation. If the epineurium of frog nerve were an effective barrier to diffusion of any solute (Feng and Liu), and in particular an effective barrier to the diffusion of ions (Hodgkin, Huxley), and if the epineurium should play an immediate role in determining the electrical

> characteristics of nerve (Rashbass and Rushton), then, all the work that has been done in the past with intact nerve trunks would stand in need of radical revision, because all the results heretofore obtained would have been vitiated by exceedingly important sources of error. Indeed, there would be in the literature on nerve physiology hardly a single important observation that could stand uncorrected. (Lorente de Nó 1950)

Hodgkin and Huxley both recall that the arguments against the sodium hypothesis before and during the war influenced their interpretation of their 1939 result; Huxley adds that the then-prevailing view that hydrated potassium ions were smaller than hydrated sodium ions, which intuitively accounted for the selective potassium permeability through a sievelike mechanism, further discouraged a serious consideration of the sodium hypothesis (HNA p. 296). Thus, when they—according to Huxley, mostly Hodgkin (HNA p. 292)—wrote a fuller report of these experiments toward the end of the war (Hodgkin & Huxley 1945), they did not raise the possibility that the overshooting action potential resulted from an increase in sodium permeability. Instead, they offered four alternative suggestions: (1) an increase in anion permeability, (2) a change in the dipole orientation of the membrane, (3) an effect of inductance (*à la* Cole), and (4) an emf or battery in series with the capacitor, rather than in parallel. Each hypothesis ended with a critique, however, revealing their skepticism about all the possibilities:

> [on anion permeability] Such a state of affairs is theoretically possible, but does not seem at all probable, since it is hard to imagine that the concentration or mobility of lactate or any other organic ion would be sufficient to swamp the contributions of K+ and Cl- to the membrane potential.
>
> [on a dipole switch] This is not an impossible assumption, although it is a little hard to imagine that such a change would leave the membrane capacity unaltered during activity.
>
> [on inductance] We are reluctant to accept the idea of a genuine inductance in the membrane, since it is difficult to attach any physical significance to such a concept.
>
> [on the series-capacity hypothesis] This hypothesis has not been developed in any detail and may not bear quantitative investigation. (Hodgkin & Huxley 1945)

Hodgkin writes that, in retrospect, both he and Huxley 'came to regret the discussion in that paper,' particularly regarding omission of the possibility of a transient selective permeability to sodium, but notes that 'things looked rather black for the sodium hypothesis both then and several years later' (C&D p. 252). In later years, Cole also referred to his own initial recordings of intracellular action potentials with Curtis, acknowledged the error in the 1942 paper, and commented on his colleagues' response to it:

> Our action potentials (Curtis and Cole 1940) were quite variable and inconclusive but we soon had word from Hodgkin and Huxley that they had done much the same thing, at about the same time and probably for much the same reason, but much better. . . . These results we fully confirmed (Curtis and Cole 1942) except for the published action potential of 168 mV which I came to believe was probably the result of an overcorrection for the electrode and the amplifier input capacities. When Hodgkin and Huxley were able to publish their work in full after the war (1945), they most generously spoke of their confirmation of *our* work. (MII p. 145)

Huxley recalls that his own distrust of the sodium hypothesis was finally alleviated in October 1945 by a lecture at the Royal Society given by August Krogh (1874–1949), in which he reported on radioactive tracer studies that had indicated that membranes were not as impermeable to sodium as had previously been believed. 'From then on,' he writes, 'the sodium hypothesis was under active discussion between Hodgkin and myself.' (HNA p. 295) Huxley expounds further on the Krogh lecture and its consequences in a retrospective he wrote on Hodgkin's life:

> In particular, he [Krogh] mentioned the exchange of sodium across cell membranes, contradicting the previous belief that cell membranes were completely impermeable to sodium ions. This implied the continuous activity of a 'sodium pump' extruding the sodium that entered the cell passively down its electrochemical gradient. It occurred to me that if the action of this pump were temporarily interrupted,

> sodium ions would continue to enter, tending to cause the interior to go electrically positive, and that this might be the origin of the overshoot. When I mentioned this idea to Hodgkin, he immediately pointed out that it was totally inadequate because, if the rate of entry of sodium ions were sufficient to cause the known rapid rise of internal potential in an action potential, the energy required to expel the sodium that would be entering continuously at rest would be far more than could be provided by the known oxygen consumption of nerve. So we began to discuss the related hypothesis that the overshoot was due to the increase in membrane permeability postulated by Bernstein being highly specific for sodium ions. (Huxley 2000)

Interestingly, as far back as 1902, Ernest Overton (1865–1933) published a paper entitled *Über die Unentbehrlichkeit von Natrium- (oder Lithium-) Ionen für den Contractionsact des Muskels (On the indispensability of sodium or lithium ions for muscle contraction).* As the title indicates, Overton reported that muscles did not twitch without extracellular sodium. This discovery, however, was somehow lost from the stream of science that influenced early neurophysiology and was not recovered until after publication of the Hodgkin-Huxley 1952 series of papers. Indeed, Huxley states that, had he and Hodgkin been aware of Overton (1902), they would likely have considered the sodium hypothesis more seriously much sooner (HNA p. 292).

After the war, in late 1945, Hodgkin and Huxley restarted experiments in Cambridge on crab axons, as squid were locally unavailable. Their thinking about the basis of excitability, and about physiology in general, had changed. Hodgkin writes in his memoir,

> I found it much harder to give tutorials in Trinity College than before the war. This was partly because I had forgotten a good deal and partly because I had ceased to believe in some of the principles that had once seemed to hold physiology together. The constancy of the internal environment remained as important as ever, but the ways in which constancy was achieved had become more complicated. It was also clear that much that I had read and taught before the war had been wildly oversimplified, if not downright wrong. . . . I suppose that after five years working as a physicist I had little use for biological generalizations and always wanted to concentrate on the physicochemical approach to physiology. This didn't go down well with most medical students. (C&D p. 262)

Among their changes in perspective, as noted, was the willingness to reconsider which ions permeated the membrane, and under what conditions. Through the following year, they studied the changes in potassium permeability of crab axons during activity. Hodgkin found that bathing a stretch of axon in higher potassium concentrations yielded a higher conductance of the axon (Hodgkin 1947). He reasoned that the converse might also be true, such that a stimulus-evoked increase in conductance might indicate an accumulation of external potassium. Hodgkin and Huxley tested this idea by evoking trains of action potential in axons bathed in tiny amounts of fluid, so that any ions extruded from the axoplasm would not diffuse away. Indeed, the conductance was increased, leading them to conclude that potassium 'leaked' from the axon when action potentials were fired. Their thinking extended beyond the flux of potassium and, for the first time, they openly revisited the sodium hypothesis in the discussion:

> The simplest way of accounting for the leakage of potassium during activity is to assume that the nerve membrane becomes temporarily permeable to sodium or to one of the internal anions. (Hodgkin & Huxley 1947)

They proceed with a rough calculation based on the idea that an increase in sodium permeability drives a potassium efflux as various equilibria are restored, but which ends with the comment:

> The preceding argument is put forward only because it is the simplest qualitative explanation of the facts. There are several reasons for believing that the true situation is more complicated. (Hodgkin & Huxley 1947)

The retrospectives provide an interesting addition to the even-toned voice of the papers. Hodgkin writes that he and Huxley spent the winter of 1946–1947 coming up with possible mechanisms for the action potentials they had recorded. The sodium theory was high on their list, although the means by which sodium would cross the membrane was a mystery. Their best guess was that a carrier system—molecules with negative charges or dipoles that would attract sodium ions—might shuttle the ions across the membrane. During his war service, Huxley had cultivated a great facility with numerical methods, computed on mechanical Brunsviga calculators (see Appendix 5), and when research slowed and then stopped owing to an unusually severe British winter, exacerbated by shortages of both food and coal, he used the time away from the lab literally to crank out, with mittened hands, theoretical waveforms of propagating action potentials (C&D p. 269–271). Hodgkin recalls,

> In these theoretical action potentials the reversed potential difference at the crest of the spike depended on a selective increase in sodium permeability and a low internal concentration of sodium ions. Huxley felt all along that this was a likely mechanism, but I was more doubtful, partly because there seemed to be quantitative discrepancies, and partly because I hankered after a mechanism which would give a transient reversal, so accounting for repolarization, oscillations, and the transient nature of the action potential. We tried various mechanisms that I thought might operate in this way, but Huxley shot them all down, leaving a rise in permeability to sodium ions, or perhaps to an internal anion, as the most likely cause of the reversed potential. (C&D p. 269–270)

In other words, Huxley saw that sodium permeability was necessary, but Hodgkin knew that the hypothesis, as it stood, was insufficient.

It is also notable that Hodgkin, unlike Huxley, hesitated to discredit the report of his former collaborators, Curtis and Cole, regarding the insensitivity of the action potential to the removal of external sodium. Even though he himself had recorded data to the contrary, Hodgkin apparently did not dismiss the Americans' work until he heard of the results obtained by his pre-war friend and scientific colleague, Bernard Katz.

Hodgkin first met Bernard Katz (1911–2003) early in his research career. Their work had converged, as both had found evidence for nonpropagated, subthreshold responses in crab axons, an observation that seemed to conflict with the all-or-none theory of excitability (Katz 1937; Hodgkin 1938) and which—like the local circuit theory—was met with doubt by Erlanger and colleagues in America. Although Katz worked in the group of A.V. Hill (1886–1977) at the University College London (UCL), rather than in Cambridge near Hodgkin, the two young scientists continued to discuss their work regularly and interacted directly at Plymouth before the war. In a letter to his mother in July 1939, Hodgkin wrote,

> Katz, a refugee who works on nerve, has been down here for a few days, and I have seen a good deal of him. He is going to Australia in a fortnight to work with Eccles in Sydney. He is a very good person to talk science with. (C&D p. 132)

Katz, who was born of a Russian-Jewish family in Germany and who became stateless upon fleeing to Britain, indeed went to Australia. After the war broke out, he obtained British citizenship and served in the Royal Australian Air Force (as a radar officer). After the war, he returned to UCL, where he remained for the rest of his life. Hodgkin writes,

> Towards the end of 1946, Bernard Katz sent me a manuscript in which he showed, among other things, that crab axons became inexcitable in salt-free sugar solutions (Katz 1947). As this agreed with my own experience I began to think that Curtis and Cole's (1942) result must have been wrong and that there was hope for the sodium theory. (C&D p. 270)

When Hodgkin finally was able to return to experiments on the squid axon at Plymouth in the summer of 1947, Huxley was absent for the simple reason that he was getting married. Ironically, therefore,

Hodgkin's direct tests of the sodium hypothesis were ultimately done without Huxley. Hodgkin initially worked alone, but Katz joined him in the autumn. They recorded action potentials from squid axons with the intracellular recording technique that Hodgkin and Huxley had worked out before the war and measured the effects of changing the extracellular concentration of sodium ions. The introduction to the paper—which appeared more than a year later, in March 1949, owing to publication delays—begins with an assurance that the action potential overshoot is real, and includes an appropriate nod to Huxley:

> [T]here is now little doubt that the membrane potential of certain types of nerve fibre does undergo an apparent reversal which cannot be reconciled with the classical form of the membrane theory. Several attempts have been made to provide a theoretical basis for this result (Curtis & Cole, 1942; Hodgkin & Huxley, 1945; Höber, 1946; Grundfest, 1947), but the explanations so far advanced are speculative and suffer from the disadvantage that they are not easily subject to experimental test. A simpler type of hypothesis has recently been worked out, in collaboration with Mr. Huxley, and forms the theoretical background of this paper. (Hodgkin & Katz 1949)

The simple, testable hypothesis of sodium permeability is then explained in a straightforward fashion, but acknowledges that the mechanism of such permeability remains unknown:

> According to the membrane theory excitation leads to a loss of the normal selectively permeable character of the membrane, with the result that the resting potential falls towards zero during activity. This aspect of the theory is at variance with modern observations and must be rejected. However, a large reversal of membrane potential can be obtained if it is assumed that the active membrane does not lose its selective permeability, but reverses the resting conditions by becoming highly and specifically permeable to sodium. The reversed potential difference which could be obtained by a mechanism of this kind might be as great as 60 mV. in a nerve with an internal sodium concentration equal to one-tenth of that outside. The essential point in the hypothesis is that the permeability to sodium must rise to a value which is much higher than that to potassium and chloride. Unless this occurs the potential difference which should arise from the sodium concentration difference would be abolished by the contributions of potassium and chloride ions to the membrane potential. The hypothesis therefore presupposes the existence of a special mechanism which allows sodium ions to traverse the active membrane at a much higher rate than either potassium or chloride ions.
>
> A simple consequence of the hypothesis is that the magnitude of the action potential should be greatly influenced by the concentration of sodium in the external fluid. Thus the active membrane should no longer be capable of giving a reversed e.m.f. if the external sodium concentration were made equal to the internal concentration. On the other hand, an increase of membrane reversal would occur if the external sodium concentration could be raised without damaging the axon by osmotic effects. (Hodgkin & Katz 1949)

The predictions were fulfilled, and the data provided convincing, if indirect, evidence that the upstroke of the action potential depends on sodium. Hodgkin and Katz therefore concluded that the membrane indeed must become permeable to sodium at the time of the action potential, which would account for the change in transmembrane voltage. To express this idea quantitatively, Hodgkin and Katz built on the work of Goldman (1943), which assumed that the electric field was constant through the membrane (hence the name 'constant-field theory') and that ions could—somehow—diffuse through a lipid membrane the way they diffused in aqueous solution. They derived an equation that defined how the transmembrane voltage depended on the relative permeabilities of sodium, potassium, and chloride. With this equation, now known as the Goldman-Hodgkin-Katz voltage equation, they estimated that the resting membrane was twenty-five times more permeable to potassium than to sodium; during the action potential, the permeability ratio switched almost completely, so that sodium was twenty-fold more permeant. Permeability, however, which had the simple units of a rate—cm/sec—remained a physically enigmatic quantity.

Further analysis of the results gave extra information: the rate of rise of the action potential was proportional to sodium concentration. Drawing on simple relations between the capacitance and the rate of voltage change, Hodgkin and Katz were able to calculate, for the first time, the total inward ionic

current carried by sodium during the upstroke of the action potential. Nevertheless, this transmembrane sodium flux was still only inferred, and a number of phrases in the paper, including the 'special mechanism' invoked in the introduction, still carry the skepticism initially voiced by Hodgkin:

> [I]t is much more difficult to accept the assumption that the active membrane can become selectively permeable to sodium. We therefore suggest that sodium does not cross the membrane in ionic form, but enters into combination with a lipoid soluble carrier in the membrane which is only free to move when the membrane is depolarized. Potassium ions cannot cross the membrane by this route, because their affinity for the carrier is assumed to be small. An assumption of this kind is speculative but not unreasonable. . . .
>
> . . . The experiments described in this paper are clearly consistent with the view that the active membrane becomes selectively permeable to sodium, and thereby allows a reversed membrane e.m.f. to be established. The evidence is indirect, and the sodium hypothesis cannot be pressed until more is known about the ionic exchanges associated with nervous transmission. (Hodgkin & Katz 1949)

Clarity about these ionic exchanges could only be achieved by a direct measure of the purported sodium current. Obtaining such data, however, promised to be difficult. First, the accumulating evidence that sodium permeability—however mysteriously it might be initiated and accomplished—would be associated with inward sodium current on action potential upstroke imposed a complicating biological twist onto Ohm's law. In a simple ohmic situation, current, I, is equal to voltage, V, divided by resistance, R. Plotting current as a function of voltage therefore would give a straight line with a slope of $1/R$; since resistances cannot be negative, the slope would be positive. A sodium current that was tiny at the resting potential but large as the voltage approached zero millivolts would create a region of negative slope—an unstable situation associated with positive feedback—which was not easily measurable. Second, the variables were not clearly limited to current, voltage, and resistance. Hodgkin and Cole, together and separately, had spent years describing the basic electrical properties of the axon, Cole pursuing an equivalent circuit including capacitance and inductance, and Hodgkin analyzing local circuit currents along the length of the axon. Their and others' work illustrated the complexity of 'cable properties' and the associated equations that quantified the relationships among factors controlling the temporal change and spatial spread of voltage. Given that action potentials propagate, any measurement of current would have to contend not only with the instability of current as a function of voltage, but with the variation of current as a function of distance.

The first problem, of unmeasurable variables under unstable conditions, might be overcome by some sort of negative feedback to counteract the positive feedback; the second, of spatial variations in voltage, might be resolved by preventing propagation by making the entire axon generate an action potential at once. In summer 1947, working alone at Plymouth on the effects of sodium on squid axon action potentials, Hodgkin wrote to Cole proposing some experiments to achieve such spatial control, which they might undertake jointly during Hodgkin's upcoming trip to America:

> I am also interested in the possibility of stimulating an axon with a diffuse electrode in such a way that the axon is excited uniformly over a length of one or two centimetres. This might give useful information about the nature of the active process uncomplicated by propagation and local circuits. What are your plans and views? (C&D p. 281)

Cole replied,

> I am sure that you will be excited to hear that we spent the whole summer with an internal electrode 15 millimetres long and about 100 microns in diameter . . . The two principal ideas are first the use of the central outside region with a guard region on each side, and second the use of a feedback circuit to control either the current flow in the central region or the potential difference in that region to the desired value. (C&D pp. 281–282).

Cole describes this setup more picturesquely in his retrospective:

> As a phenomenological description, it could be said that the axon had been robbed of its ancient right to propagate an impulse by eliminating the local circuit currents, $\partial^2 V/\partial x^2$, by which an active region normally reached ahead to move itself along the axon. (MII p. 244)

Regarding the question of feedback control—the essence of the voltage-clamp technique—Cole further explains that such methods had become pervasive during the technological advances associated with the war:

> The control concept had been highly developed during World War II, principally with feedback electronics. It was widely applied afterward. . . . In general the difference between the actual and the desired position of a system is used to control the power to reduce this error. (MII p. 246)

Hodgkin, too, was considering feedback methods soon after the war, as Huxley explains in his autobiography:

> Both Hodgkin and Cole suspected that the all-or-none character of the nerve action potential was due to a current-voltage relation in the membrane that was continuous but included a region of negative slope which caused positive feedback and therefore instability. Such a feature would make it difficult to measure the current-voltage relation. I remember a discussion with Hodgkin, probably in 1945, in which he pointed out that it would be necessary to use electronic feedback to an internal electrode so as to control the internal potential ('voltage clamp') and to make it undergo stepwise changes. I replied that it would be just as good to feed current from a low-impedance source, but Hodgkin had realized that this would be an imperfect arrangement since the electrode would become polarized by the high current density that would be needed. (HNA p. 297)

As it turned out, Hodgkin and Cole did not have the chance to collaborate during the former's visit in spring 1948, but they did meet and discuss their science. Hodgkin told Cole of his results with Katz on sodium entry into the axon on the upstroke of the action potential, and Cole and his then-collaborator George Marmont showed Hodgkin their initial work with feedback control. Marmont, who remained focused on the idea that conductance changes were dependent on *current,* succeeded in supplying current feedback through a single electrode and recorded what would now be called escaping spikes. These current responses resembled delayed, inverted action potentials, resulting from an initially stable experimental 'clamp' that was gradually overridden by large ionic currents (Marmont 1949). Cole worked separately on a modification of the same setup and attempted to control, or 'clamp,' the membrane voltage. The traces he later published showed that depolarization of the membrane was associated with an inward followed by an outward current, but the waveforms were too distorted to withstand quantitative analysis (Cole 1949). Hodgkin provides a recollection that is fairly generous:

> [Cole and Marmont] showed me the results they had obtained the previous summer at Woods Hole with the membrane current or potential of a giant nerve fiber under the control of electronic feedback. I gathered that Marmont was more enthusiastic about current control and that, perhaps for this reason, they had not done many experiments with voltage control. However, the results which Cole showed me clearly illustrated the essential features of records obtained with the 'voltage-clamp' technique. (C&D p. 282)

Huxley, however, recounts that Cole had, in fact, run into the problems anticipated by Hodgkin:

> Cole, together with Marmont, was the first to make experiments of this type [feedback control] on the squid giant fiber in the summer of 1947 (Cole, 1949). However, their experiments were limited: Marmont had originally devised the apparatus with the intention of controlling the membrane current and Cole had made an addition which made it possible to use it to control the internal potential. Using it in this voltage-control mode, they did show that the current-voltage relation is continuous with a region of negative slope (Cole, 1949), but they did not analyze the current into components carried by different ions; further, their apparatus was not a true voltage clamp since they controlled the current by feedback

> from the same internal electrode by which current was injected. This effectively provided a low-impedance source from which potential changes were applied to the internal electrode and the results were therefore distorted by electrode polarization, as Hodgkin had foreseen: the long-lasting outward current during what should have been a constant raised internal potential declined because the potential of the axoplasm did not follow perfectly the potential applied to the wire. (HNA p. 298)

Cole, too, later commented on the shortcomings not only of the technique but of his conceptual framework at the time, which limited his ability to interpret the results:

> The early inward current flowing against the resting potential had come to be expected, but again I was greatly disappointed not to find a steady state negative resistance. Even though the extent of my ignorance and confusion was more clearly revealed, I was very pleased by the direct records of the amplitude and form of the currents. They gave good basis for at least a qualitative explanation of the initiation, rise, and recovery of the action potential and its propagation (Cole 1949). (MII p. 259)

Indeed, although the flow of technical information from Cole to Hodgkin is recalled in most of the retrospectives and biographies, the flow of conceptual information in the opposite direction is less strongly emphasized. It is clear that Cole showed Hodgkin his proto-voltage clamp setup and experiments early 1948, but at the time Cole presumably was still convinced of the results from his 1942 paper with Curtis, which appeared to rule out a primary role of sodium ions in generating the action potential. Cole apparently did not read Hodgkin and Katz's results as offering an explanation of his own recordings and instead remained focused on current as the independent variable. He writes,

> [Hodgkin] could not convince me that my data had any other interpretation than an inward current arising from a linear small outward current. (MII p. 268.)

Cole then explicitly recounts that he argued strongly against a carrier model when Hodgkin showed him his own yet-unpublished results with Katz from the previous summer (MII p. 269). The record is silent, however, regarding how (or whether) Cole or Marmont might have tried to account mechanistically for the inward currents they had recorded, before or even after Hodgkin provided them with evidence that the action potential upstroke was associated with a membrane permeability to sodium ions.

It was the conceptual framework built up by Hodgkin and Huxley that made the next phase of their experiments—the one that provides the basis for the five classic papers of 1952—proceed rapidly and smoothly. Huxley recounts that Hodgkin had begun to consider the novel idea that permeability was sensitive to *voltage* itself, based on his early, contentious work on subthreshold responses in crab axons:

> The prewar experiments [Hodgkin 1937c] in which Hodgkin had seen the local responses of crustacean nerve fibres when stimulated by a shock just too weak to start a full-sized impulse had led him to believe that the increase in permeability during the action potential was not itself an instantaneous change but was graded with the change in internal potential. As the internal potential was raised the increase in permeability (even if it allowed all species of ions to enter or leave, as supposed by Bernstein) would tend to raise the internal potential so that a point could come at which the situation was unstable: any small rise in potential would cause a permeability increase that would cause an additional potential rise, and so on in an explosive manner until a new equilibrium was reached. This instability would be the cause of the all-or-none character of the action potential. Hodgkin [in 1946] conceived an experiment in which current was passed between a long wire in the inside of a nerve fibre and the external solution; the potential of the interior would be monitored and a feedback amplifier would control the current so that the potential underwent a predetermined time course. This arrangement is referred to as a 'voltage clamp' because it is usually used to bring the internal potential from its resting level to another level and to 'clamp' it at this level for a substantial period. (Huxley 2000)

In the summer of 1948, Hodgkin returned to Plymouth to resume experiments on squid axons and to pursue a method of voltage control that overcame the polarization problems of single electrode-mediated feedback. Hodgkin and Katz developed the double spiral electrode; Huxley, who arrived six weeks later, began to build the circuit Hodgkin had previously sketched out. They obtained some initial

recordings, which they presented at a conference in Paris the following April, some months before Cole's initial voltage-clamp study was published in October. In summer 1949, Hodgkin and Huxley returned to Plymouth to continue their research:

> At first squid were in poor supply and we took a few weeks to get going. But by mid-July 1949 Katz had joined us, there was a good supply of living squid and in the next month we obtained virtually all the voltage-clamp records that we used to illustrate the papers published in 1952. I believe we were able to do this quickly and without leaving too many gaps because we had spent so long thinking about the kind of system which might produce an action potential similar to that in nerve. We also knew what we had to measure in order to reconstruct an action potential. (C&D p. 289–290)

Katz was involved in the early experiments but soon turned his attention to muscles, apparently for aesthetic reasons. In a twenty-first century interview, his collaborator, Paul Fatt (1924–2014), recalled the summer of 1948:

> I went to Plymouth and there they were, Katz and Hodgkin and Huxley, the three of them, working with squid axons. . . . And somehow I got attached to Katz because he wasn't happy with all of this, he liked to see action potentials and here they are suppressing them; they're actually stumping them, they won't have action potentials. He liked action potentials. And he wasn't going to be on this analysis. . . . [T]hey were all worried about not being able to get squid. Getting squid and dissecting it. Oh, Katz I think, was dissecting them; that's the only thing he was doing because he didn't like this no action potentials. (Fatt 2013)

After rapidly gathering data that summer, Hodgkin and Huxley took two years to analyze and write the five resulting papers; four were submitted in October 1951, appearing in print in April of the following year, and the fifth, which included the computational analysis, was submitted in March 1952 and published in August. Although the completed opus did not provide the molecular explanations that Hodgkin and Huxley had hoped for—which Hodgkin describes as 'initially a disappointment' (C&D p. 291)—the results offered solid, quantitative evidence for a series of revolutionary insights.

Revolutions, however, are rarely accepted without opposition. In June 1952, Hodgkin attended a symposium at the Cold Spring Harbor Laboratories where he presented the work he had recently completed with Huxley and Katz. Hodgkin's brief report of the meeting in his memoir savors of understatement:

> At all events I had to work hard for the privilege of being there. After my talk, someone, possibly Ralph Gerard, organized an evening session with Cole and perhaps a dozen nerve people there and cross-questioned me step by step on the details of our five papers; this took several hours. (C&D p. 324)

Regardless of who accepted or even fully grasped the ideas discussed that evening, Hodgkin and Huxley had largely solved the more-than-a-century-old puzzle of the basis of bioelectricity. They had demonstrated that sodium and potassium ions flow across the membrane, not shuttled by carriers, but diffusing through voltage-sensitive, time-dependent, ion-selective, conductance-resembling pathways, which would later be molecularly identified as ion channel proteins. These extraordinary results, their precise quantification, and their exquisitely multifaceted interpretation—acknowledged by a Nobel Prize to Hodgkin and Huxley in 1963—accounted for virtually all the key observations of the field, including the action potential itself. The discipline of neurophysiology entered a new era.

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