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Models and instruments in the development of electrophysiology, 1845-1912

1. INTRODUCTION

THE NINETEENTH CENTURY witnessed the transformation of physiology from a discipline characterized by theories based on qualitative, descriptive accounts derived mainly from indirect sources, such as comparative anatomy, into a discipline characterized by theories based on controlled experimental investigations of processes transpiring directly within the organism. Two lines of inquiry helped to bring about this transformation of the field. Some physiologists, particularly the French school of Magendie and Claude Bernard, sought to obtain operative control over the functioning of the organ through vivisection and the application of chemical or physical means. Others, particularly German physiologists, attempted to follow the course of physical and chemical changes during the functioning of isolated organs by means of measurements by appropriate instruments. The expressed objective of this second approach to physiology was the development of quantitative theories of the chemical and physical processes underlying specific organ function. Work in electrophysiology, particularly the research a forts spanning the period between 1845 and 1912, was the most successful example of this approach.

Work in electrophysiology dates from the publication of Galvani's researches on decapitated frogs in 1791 and the ensuing debate with Volta over the nature of "animal electricity." Their investigations were deepened and extended by Humboldt, Ritter, Nobili, Matteucci, and others. The results were qualitative and phenomenological. Not until the work of Emil DuBois-Reymond and Hermann Helmholtz

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The following abbreviation is used: *UTE*, Emil DuBois-Reymond , *Untersuchungen über thierische Elektrizitkät* (3 vols., Berlin, 1848-1849).

beginning around 1845 did the field begin to be guided by the explicit purpose of formulating a quantitative theory of nerve and muscle action sustained by exact measurements. Nevertheless, although this objective was vigorously pursued, it was not achieved until 1912, when Julius Bernstein (1839-1917) published the mature version of his membrane theory of the action potential, the first truly quantitative theory in electrophysiology.

Bernstein first outlined his theory in 1902. He proposed that each nerve cell and muscle fiber is surrounded by a membrane separating the cell interior from the fluids of the extracellular medium. Bernstein supposed that owing to the semi-permeability of the membrane to different species of electrolytes, different concentrations of electrolyte would .build up on the inside and outside of the membrane, giving rise to an electrochemical potential. He argued that a positive concentration gradient (from inside the cell to out) of potassium ions renders the interior of nerve and muscle cells electronegative with respect to the exterior. When stimulated, the membrane alters its permeability, leading to the opening of numerous "pores" that permit an influx of other ions, such as sodium and chloride ions, with the result that the cell rapidly depolarizes. The resulting flow of current could be detected by sufficiently sensitive instruments and registered as the "action current" *(Aktionsstrom)* of the nerve or muscle. Bernstein argued that the action current is the key event in understanding nerve transmission and the onset of the chemical changes leading to muscle contraction.

Bernstein's theory was based explicitly on the improvement and modification of an earlier theory proposed by his teacher, Emil DuBois-Reymond , who had devised ingenious demonstrations to make plausible the claimed electrical nature of nerve transmission and muscle contraction. DuBois-Reymond could not demonstrate this claim unequivocally, however, and much of the ensuing discussion centered on demonstrating that the effects detected by DuBois-Reymond 's instruments were not artifacts but actually representative of the underlying physiological processes themselves. Bernstein was the first to overcome this difficulty and to demonstrate convincingly the electrical nature of nerve transmission and the onset of muscle contraction. Bernstein succeeded by modifying DuBois-Reymond 's theory and assimilating to it aspects of several other lines of inquiry in nerve-and-muscle physiology and in physical chemistry, particularly the ion theory of Nernst and Ostwald.

In this paper I discuss the role of models, formal analogies, and refinements in instrumentation in transforming the first vague hunches and qualitative accounts concerning the function of bioelectric currents manifested during nerve and muscle action into the first mature theoretical account of the domain, the membrane theory. I especially want to call attention to the deep interdependence between the refinement of instrumentation, the construction of models, and the evolution of the conceptual and theoretical structure of the field. The instruments a ed for detection of bioelectric currents in nerves and muscles were significant not just in the trivial sense that they made it possible to explore the key phenomena of the domain, but more importantly, tie instruments themselves sometimes suggested, and on occasion even functioned as explanatory models for the phenomena under investigation. Models provided crucial intermediary structures for bridging between rudimentary theoretical accounts and their improved offspring. They did so by establishing analogies to other, better understood areas, particularly electricity and magnetism in the early phases of the development of the field and to physical chemistry during the mature phase of theory building.

Four different types of models occur in our history. One is a simulacrum, a physical model representing the presumed structure of nerves, muscles, and membranes. A second type, more common in our history tan simulacra, are what Peter Achinstein calls analog models.[1] An analog model does not reproduce the characteristics of the prototype, but establishes correspondences on similarities in the relations of respective parts and structures. The analog model is not intended to b a true description of the prototype: it need not reproduce all the characteristics of the prototype but only certain salient features considered relevant.

A third type of model is what Norton Wise has called a formal analogy. More speculative than analog models, formal analogies are heuristic guides useful for exploring and depicting a framework of relations that future theoretical account ought to incorporate. Such models depict a similarity between the formal structure of some known or imagined system and the relations discerned to hold among the principal phenomena of the object domain under investigation. Pflüger considered the conditions controlling the activity o the nerve to be formally analogous to the conditions controlling equilibrium of a piston driven in a cylinder by a spring and constrained in its motion by a column of water. The intent of this formal analogy was not to depict a prototype of nerves and muscles; nor was its intent to establish analogies suitable for applying some specific theory from another domain to the investigation of

[1] Peter Achinstein, *Concepts of Science* (Baltimore: Johns Hopkins University Press, 1968).

nerves and muscles. Rather its sole purpose was for depicting the structural relations among the phenomena of nerves in a form that might suggest a basis for developing a theory in terms of the broad principles of energetics.

Like formal analogies, theoretical models do not refer to the object under investigation, such as a nerve or muscle, but to the *set of assumptions* about the object domain. A theoretical model offers a mechanism or internal structure-in the case of neurobiology, a micro-structure-that explains the behavior and properties of the object or system. Furthermore, a theoretical model attempts to embed the object domain under investigation in a broader framework of theories considered to be more basic or fundamental. In the cases I will explore, the theoretical models constructed by DuBois-Reymond and Bernstein function as interpretive schema for applying theories of electromagnetism and physical chemistry to the domain of neurobiology.

Although theoretical models need not be quantitative, quantitative theoretical models played a special role in the development of theories concerning bioelectric currents. Julius Bernstein's attempt to model physiological membranes in terms of analogies to the ion-sieves employed in physical chemistry and thermodynamic discussions enabled him to establish quantitative relationships between several parameters subject to experimental determination. The quantitative model established for the first time a means of identifying causal relationships linking the principal phenomena of the domain. This in turn suggested a more refined picture of the fundamental entities to be employed in a theoretical account of the origins of the action potential and its role in nerve and muscle action.

2. THE FARADAY OF PHYSIOLOGY

DuBois-Reymond 's contributions to neurophysiology were based on his pioneering efforts in the construction of what in his own day were a new generation of scientific instruments.[2] Throughout his career DuBois-Reymond concentrated on the development and refinement of two types of instrumentation: electrodes for conducting weak bioelectric currents without distortion and instruments for detecting and amplifying those currents. Previous efforts to prove the electrical nature of neural transmission had failed because currents

[2] DuBois-Reymond's contributions to neurobiology are well treated by Christoph von Camphausen, "Elektrophysiologie and physiologische Modellvorstellungen bei Emil DuBois-Reymond," in Gunther Mann, ed., *Naturwissen und Erkenntnis im 19. Jahrhundert: Emil DuBois-Reymond* (Hildesheim: Gerstenberg Verlag, 1981), 79-104.

produced by the surface contact of metal electrodes with the fluids in nerve and muscle tissues could not be eliminated.

In his earliest researches, DuBois-Reymond attempted to avoid the problems that had confronted his predecessors by making contact with nerves and muscles with electrodes constructed from numerous (144) layers of Swedish filterpaper soaked in saline solution (see figure 1).

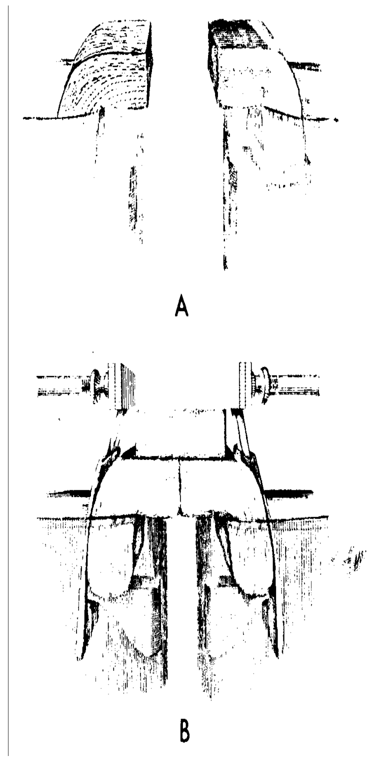

FIG. 1 DuBois-Reymond 's filterpaper electrodes. In A, stacked sheets of Swedish filterpaper soak in saline solution. B adds zinc electrodes. DuBoisReymond, *UTE*, *1* (1848).

These were laced in a glass vessel containing a 0.6% saline solution. Silver electrodes were dipped into the saline bath without direct contact with the filterpaper. In order to be sufficiently sensitive to the weak currents of nerve and muscle preparations, the filterpaper electrodes had to have a large cross section. This placed limitations on the sort of recordings DuBois-Reymond could make. For example, it precluded the possibility of investigating the properties of polarized nerve segments between two sources of direct current applied close to one another, a phenomenon associated with electrotonus.

A second difficulty that had frustrated previous investigations was the amplification of weak bioelectric currents to a degree sufficient for investigating their relationship to nerve and muscle action. DuBois-Reymond solved this problem by constructing a more sensitive version of the multiplier, or "astatic galvanometer" as it was frequently called, originally designed by Nobili[3]. In DuBois-Reymond 's instrument (figure 2) a conducting wire was tightly coiled about two parallel plates. Two magnetized needles, their poles facing in opposite

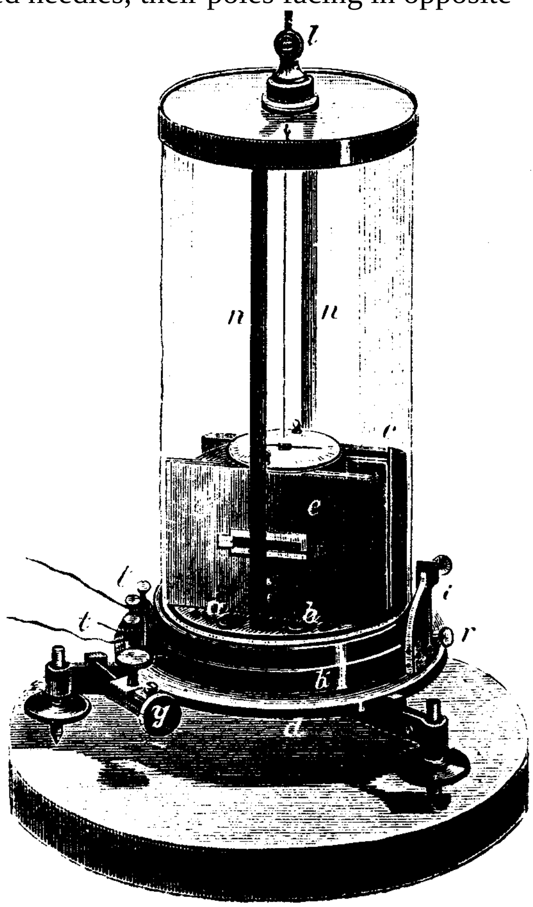

FIG. 2 DuBois-Reymond 's multiplier. The conducting wire is coiled some 24,160 times, about the places e. The upper needle is shown suspended by a thread from 1; the lower swings in the plane marked by the slit in e. Current enters the instrument at t. Elie de Cyon, *Atlas zur Methodik der physiologischen Experimente and Vivisectionen* (Grieben, 1876), table xliv.

[3] *UTE, 1* (1848), 160-250.

directions to compensate for the earth's magnetic field, hung by a thread so that one needle was above, the other within, the space separating the two parallel sets of coils. Slits cut in the plates allowed the interior needle to rotate freely. The coil multiplied the magnetic effect of a weak current flowing into the instrument enough to turn the lower needle.

These instruments placed strict limits on the range of questions DuBoisReymond and could explore directly. Not only were intracellular readings impossible with his electrodes, but even with improved nonpolarizing electrodes, introduced by DuBois-Reymond in 1859. DuBois-Reymond could not take readings from individual cells at once. No direct inferences could be drawn from the activities of single nerve or muscle cells. Secondly, though sensitive by comparison with other recording devices of the same generation, the multiplier was slow for electrophysiological purposes. It could not respond to rapid changes in current intensity; it could not register the firing of single nerve or muscle cells. In later years DuBois-Reymond returned frequently to the problem of improving the responsiveness of galvanometers.[4]

Armed with his recording device, DuBois-Reymond set to work to detect the presence of bioelectric currents in nerves and muscles. His first success served as the basis for his doctoral dissertation in 1843.[5] It was a demonstration of the so-called "frog current," which had first been detected by Nobili. With his more sensitive galvanometer DuBois-Reymond and confirmed the presence of a continuous weak current flowing between the nose and tail of the frog. This effect is of no significance for modern physiology, and may have no physiological significance at all.[6] It is an artifact, but it became a central component of all of DuBois-Reymond 's later theorizing.

Turning his attention to the chief objects of his investigations, the large sciatic nerve and the gastrocnemius and sartorius muscles of the frog, DuBois-Reymond detected two phenomena exactly parallel to his *Froschstrom.* He called them the "muscle current" and "nerve current," and he took them to be "fundamental phenomena" of neurophysiology.[7] He showed that in both muscles and in nerve segments

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[4] DuBois-Reymond, "Über aperiodische Bewegung gedämpfter Magnete," Akademie der Wissenschaften, Berlin *Monatsberichte*, 1869, 807-852. He continued these investigations in three additional papers, all bearing the same title, in ibid., 1870, 537-570; 1873, 748-764; and 1874, 767-790, reprinted in Emil DuBois-Reymond, *Gesammelte Abhandlungen zu allgemeinen Muskel and Nervenphysik,* (2 vols., Leipzig, 1875-1877).

[5] DuBois-Reymond, "Vorläufiger Abriss einer Untersuchung über den sogenannten Froschstrom und uber die elektromotorischen Fische," *Annalen der Physik and der Chemie,* 58 (184), 1-30.

[6] Von Camphausen (ref. 2), 86-87

[7] *UTE, 1* (1848), 501.

a current flows from the exterior surface to the axial cross section of nerve or muscle fiber in such a way that the muscle or nerve is positively charged along its surface and negatively charged at the cross section (figure 3).

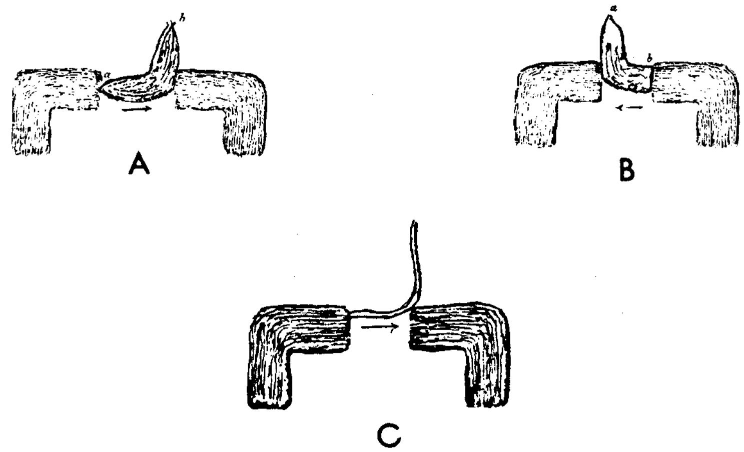

FIG. 3 The muscle and nerve currents. In A, the right-hand electrode is placed on the exterior surface of the muscle and the left-hand electrode on the tendon. The deflection of the galvanometer needle indicates a constant current from the surface of the muscle to the tendon: the exterior surface is electropositive relative to the tendon. In B, current flows from the exterior surface of the muscle fibers to the cross-sectional axis of the fibers exposed by cutting them out of the muscle. In this situation as well, the surface of the muscle is electropositive relative to the axial section. When one recording electrode is attached to the exterior surface of a nerve segment and the other to the axial cross-section of the segment, the galvanometer indicates a constant flow of current from the positively charged surface to the axis (C). DuBois-Reymond , *UTE, 1* (1848).

Important for DuBois-Reymond 's theorizing was the fact that the same manifestation of current flow from lateral surface to cross section also appeared in artificially prepared muscle segments, no matter how small the segment of muscle considered. Inspired by Ernst Brücke's microscopic investigations on the light and dark bands forming a regular pattern along skeletal muscle fibers, DuBois-Reymond aimed to show that a single muscle fiber was composed of unit "voltaic" cells manifesting the same electrical properties as the macro structures open to investigation with his instruments.

In 1866 one of DuBois-Reymond 's students, Ludimar Hermann, established that the muscle current is an artifact produced by damaging the membrane of the muscle. Because dead or injured muscle tissue is electronegative with respect to intact muscles, Hermann called this phenomenon the "demarcation current." On occasion it was referred to by the less theory-laden term, "injury current," which is its designation in modern textbooks. Quite early on in his investigations DuBois-Reymond recorded instances in muscles where he detected no "muscle current," particularly in cases where the whole muscle including its termination at the tendon was intact, but he chose to treat these as spurious effects.[8] Instead, where he found no muscle current, he argued that an opposing compensation current was set up that defeated his instruments. He took what turned out to be an artifact t be a fundamental phenomenological law of neurophysiology: that in muscles and in nerves a constant, continuous current flows from the exterior surface to the cross section.

DuBois-Reymond went on to establish a further phenomenological law relating bioelectric currents to the active state of muscles. When he tetanized a muscle by passing a series of rapid, brief electrical pulses through the connecting nerve, the muscle current registered by the galvanometer in the resting state diminished. Figure 4 illustrates the experimental set-up for calling forth the "negative variation" *[negative Schwankung].*[9] Before being tetanized with current pulses generated by the induction apparatus P, S, S, B, the muscle in its resting state exhibits a characteristic flow of current as registered by the galvanometer. During tetanus the muscle current diminishes. DuBois-Reymond called this reduction in the resting current a "negative variation." He regarded it as the central event in muscle contraction. The major objective of his program of research was to demonstrate the existence of the negative variation for nerves as well.

A leading idea informing DuBois-Reymond's work was the reductionist conviction that all phenomena in physiology could ultimately be accounted for in terms of repulsive and attractive forces acting between discrete masses. He tended to think that the fundamental phenomena underlying nerve transmission and muscle contraction are discrete in character. But, as he noted in an extended discussion of his instruments, the multiplier was incapable of distinguishing a relatively constant current from a summation of short bursts of current.[10] In order to provide evidence for his conviction that the observed steady current arose from discrete events, DuBois-Reymond devised an ingenious experiment employing what he called a "physiological rheoscope." He arranged one nerve-muscle preparation (S in figure 5) with

[8] DuBois-Reymond refers to these anomalies in ref. 5.
[9] *UTE, 1 (1* 48), 258 ff., and 2 (1849), 390 ff. DuBois-Reymond did not use nonpolarizing electrodes in 1848.
[10] Discussed in *UTE, 2* (1849), 90-93.

its nerve parallel to the muscle fibers joining the lateral surface and cross section at the tendon of a second preparation (P).[11] He showed

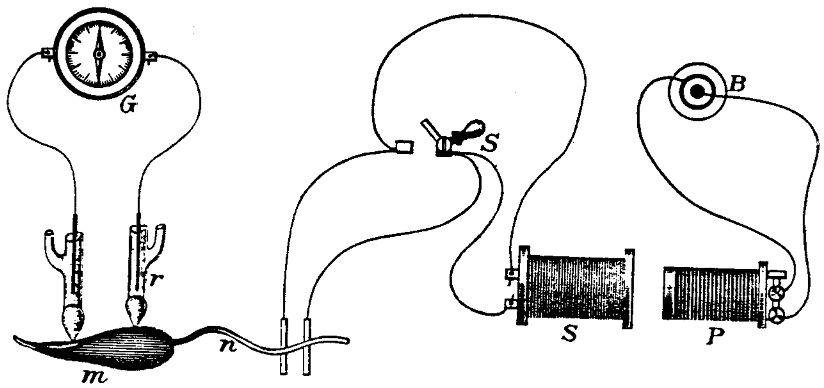

FIG. 4 DuBois-Reymond's apparatus and set-up for detecting the negative variation. Recording electrodes, r, and a galvanometer, G, are attached to the surface and tendon of the muscle m . G records the so-called muscle current. The right side of the diagram depicts the apparatus for tetanizing the muscle by a sequence of rapid induction currents: a battery, B; P, which slides within a secondary S to induce current pulses; a switch, S, which passes the induction pulses to the nerve, bringing the muscle to tetanus. During tetanus the galvanometer registers a significant reduction of the muscle current. This reduction is the "negative variation." A similar reduction of the nerve current during tetanus occurs in isolated nerve segments. Bernstein, *Lehrbuch (1900)* (ref. *40).*

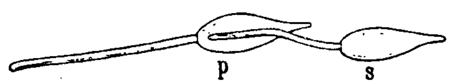

FIG. *5* The electrophysiological rheoscope. A primary muscle, P, is tetanized from the nerve. A second nerve-muscle preparation, S, with the nerve making contact between the muscle surface and the tendon (insuring that current will flow from P to S), contracts with each shock administered to P. DuBois-Reymond , *UTE, 2 (1849).*

that when P was tetanized from the nerve, S also became tetanized, and remained so until the end of the stimulation. By exciting the nerve less frequently, so that the muscle did not become completely tetanized, DuBois-Reymond found that every contraction of P was answered by one in S. But the galvanometer attached to S registered a continuous reduction in current. DuBois-Reymond concluded that each variation in the current intensity leading to a muscular contraction is accompanied by a brief, temporarily separate negative variation of the muscle current.

[11] Ibid., 87 ff.

In an accompanying diagram (figure 6), DuBois-Reymond graphically represented the way in which he viewed the relationship between the muscle current and negative variation during tetanus. The ordinate

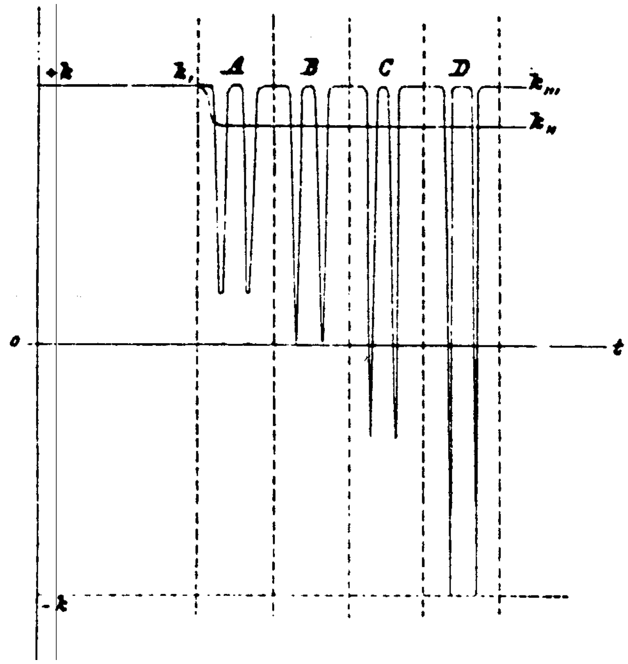

FIG. 6 Composition of the negative variation according to DuBois-Reymond. *Ok* represents he magnitude of the muscle current, *ot* time, kk_ik_{ii} the negative variation s recorded by the multiplier. DuBois-Reymond hypothesized that the negative variation is in reality a series of discrete events dependent on the discharge of numerous, miniature "voltaic cells." He noted that his instruments cold not disclose whether the magnitude of these discharges was less than (case A), equal to (case B), or greater than (cases C and D) the magnitude of the nerve or muscle current. DuBois-Reymond , *UTE 2* (1849).

ok represents the muscle current as registered by the galvanometer before contraction, and the curve k_i k_{ii} represents the reduction of the muscle current during tetanus. DuBois-Reymond concluded on the basis of the experiments with his "physiological rheoscope" that the actual shape of the curve is closer to that given by k_ik_{iii}. As the diagram indicates, he speculated that the magnitude of the negative variation might vary. Whether it extended to the abscissa, and hence was equal to he magnitude of the muscle current (as indicated in section B of the diagram), or whether it might even extend beyond the abscissa and thus possibly *exceed* the magnitude of the muscle current (as indicated 'n sections C and D of the diagram), were questions that could not be answered with the instrumentation available to him. "It is difficult to see," he concluded, "how our methods and means of measurement will ever be able to make such a determination with sufficient precision and certainty."[12]The problem of constructing recording devices sensitive enough to follow individual contractions continued to occupy DuBois-Reymond for the remainder of his career.[13] Without such instruments, he was forced to introduce speculative elements into his picture of the mechanism of muscle contraction. One of the functions of the models he constructed was to overcome deficiencies in instrumental means.

In order to support his claim that the central event of both nerve transmission and muscle contraction is the negative variation, DuBois-Reymond had to establish that the declination of the galvanometer needle in nerve preparations was also a summation of discrete electrical events. This could not be done as easily for nerves as it had been for muscles, however. The individual nerve currents were too weak and also too brief to be detected individually by the multiplier. Furthermore, no demonstration technique could be devised for nerves analogous to the physiological rheoscope for muscles, which could resolve a slow tetanus contraction into a series of discrete contractions.[14] Nerves work in an all-or-nothing fashion; the phenomenon of secondary tetanus illustrated by the physiological rheoscope does not exist in nerves. In order to silence his materialist opponents, who believed that the central event in nerve transmission was the manifestation of a *constant* current, and his vitalist opponents, who believed that the negative variation was a reduction of the *Lebenskraft* brought on by tetanus, he sought an analogue to the "physiological rheoscope" to demonstrate that the negative variation registered by the multiplier is not the reduction of a constant current in nerves, but the manifestation of many discrete electrical discharges.

DuBois-Reymond discovered a new phenomenon that he used to bolster his claim concerning the negative variation in nerves. Experimenting on an unusually long sciatic (86mm) nerve of a frog, he showed that by passing a constant current through electrodes connected to a middle segment of the nerve, the normal nerve current *increased* on the end of the segment in the direction of the applied current, and *decreased* on the opposite end (figure 7). Drawing a direct analogy to the electrotonic state employed by Faraday to explain the phenomenon of induction, DuBois-Reymond called this state of the nerve "electrotonic."[15]

[12] Ibid., 121.
[13] Ref. 4.
[14] *UTE, 2* (1849), 288-290).
[15] Ibid., 388-389.

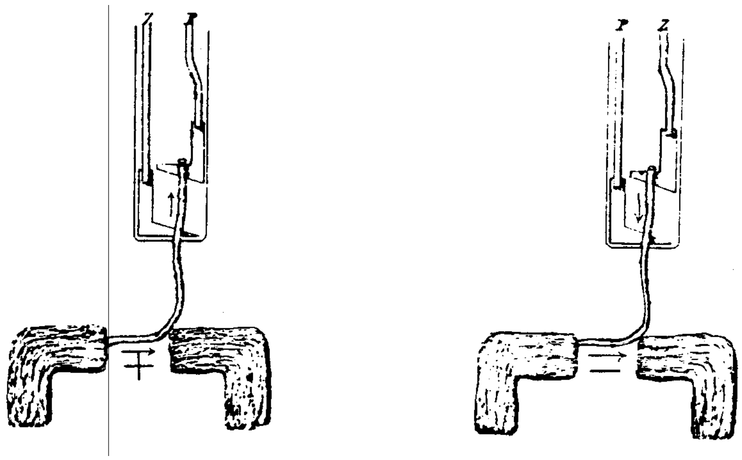

FIG. 7 The electrotonic state. (A) In the resting condition a current flows from the exterior surface of the nerve to the axial cross section, as indicated by the direction of the arrow. When a constant current is applied at the other end of the nerve, flowing from Z to P in the same direction as the nerve current, the galvanometer registers an increase in the nerve current (indicated by the + in the diagram). (B) The direction of the applied current (once again from Z to P) is reversed. In this situation the nerve current registered by the galvanometer is less than that of the rest condition (indicated by the - in the diagram). DuBois-Reymond , *UTE, 2* (1849).

Faraday had postulated that a current-carrying wire and the matter in its vicinity are put into a "peculiar condition" as long as a current continues an that a change in the condition causes current to flow.[16] Modeling his approach on Faraday's, DuBois-Reymond showed that as long as current-even current in excess of the muscle or nerve current-was applied to the nerve in a constant fashion, the negative variation did not occur. But an alteration of the applied current, by closing or breaking the circuit or by reversing the polarity of the stimulating electrodes, caused a negative variation (figure 8). Thus, DuBois-Reymond argued, the negative variation associated with nerve transmission r muscular contraction must be regarded as independent of the electrotonic state.

The relationship between the negative variation and applied current in the electrotonic state could be extended by analogy to the interpretation of the phenomena connected with the negative variation in a tetanize muscle. DuBois-Reymond drew a direct correspondence between the applied current in the electrotonic state and the constant muscle current. He considered the stimulating current-pulses

[16] .Michael Faraday, *Experimental researches in electricity,* (3 vols., London, *183955), 1,* 16-22, 69 70, 341-342, 529-530, 550.

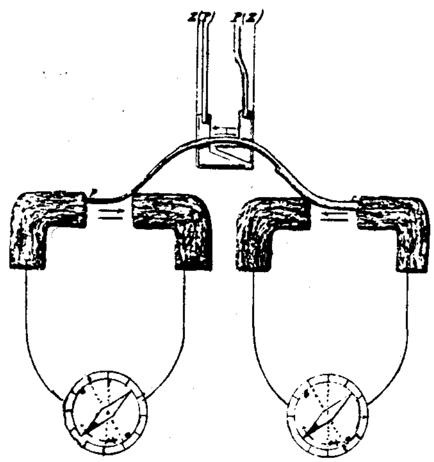

FIG. 8 Independence of the negative variation and the electrotonic state. A current enters the middle of along (86 mm) sciatic nerve. The arrows indicate the direction of current flow (the nerve current) during the rest state. As in figures 7AB, during the electrotonic state, when the applied current flows from Z to P, the nerve current through the galvanometer on the right will be less than the resting nerve current on the left (this is not indicated in the diagram); but as long as the applied current is constant, no negative variation occurs. Only by rapidly altering the applied current or by reversing the polarity, indicated in the diagram by (Z) and (P) (the arrows in the center indicate direction of current flow), does a reduction of the nerve current characteristic of the negative variation appear (indicated in the diagram by the - at both ends of the nerve. DuBois-Reymond, *UTE, 2* (1849).

producing tetanus as corresponding to the variations in the applied current intensity or reversal of polarity in the experiments on electrotonus. In both cases the negative variation of the muscle (or nerve) current was seen as an independent event produced by fluctuations in the muscle or nerve current.

DuBois-Reymond as model builder

I have discussed the phenomena and experimental demonstrations that turned out to be crucial to DuBois-Reymond's theorizing about bioelectricity and central to later discussions in the development of the membrane theory of the action potential. The three-volume work contains a multitude of other experiments, including repetitions of the work of previous researchers. In the midst of this Baconian accumulation of data, which occupies 1930 pages in all, DuBois-Reymond reports that he felt a need for some means of storing and retrieving this mass of data in a convenient fashion amenable to further exploration in neurophysiology:"[17]

[17] *UTE, 1*(1848), xii-xiii.

The number of experiments accumulated over the course of the years in such a manner that in spite of carefully kept laboratory notebooks it was impossible to have an instantaneous overview of the entire subject. On more than one occasion I repeated experiments which I had long ago performed because I had forgotten that they had already been recorded in my notebooks. In the same measure that the facts began to multiply, the network of interconnections between them, through which I hoped o fashion some unity out of the data, also began to expand and ramify.

The solution to this problem was the construction of a physical model to serve as a mnemotechnic device as well as a means for illustrating the relationships among the phenomena that DuBois-Reymond took to be central. He also expected that direct experimentation on a physical model of nerves and muscles would enable him to investigate certain phenomena that his instruments prevented him from exploring. The model represented the origin of the nerve and muscle currents by means of a copper cylinder with a galvanized layer of zinc on the outside. The cylinder hung in a container filled with an electrolytic solution. A galvanometer connected to the exterior and interior surfaces of the cylinder registered a flow of current from the positively charge later surface of the cylinder to its negatively charged cross section in analogy to the phenomena of muscle and nerve currents (figure 9).

In order to illustrate his view that each muscle can be conceived as a bundle of voltaic "cells," DuBois-Reymond put together a bundle of small, thin copper cylinders galvanized on their exteriors and submerged in an electrolytic solution (figure 10). The results he obtained with this model were in agreement with the phenomena of the muscle and nerve current.

DuBois-Reymond did not rest content with these physical models and mnemonic-devices. He attempted to go beyond them by adding an hypothesis about the underlying molecular reality. Here he relied on analog models to interpret nerve and muscle action in terms of Faraday's an Ampère's theories of electricity and magnetism. I have already noticed DuBois-Reymond's use of Faraday's work on induced current as an interpretive schema for his experiments on the negative variation. In order to apply Faraday's work to nerve transmission and muscle action, it was essential, in DuBois-Reymond's mind, to couple variation in current intensity of the nerve with induction of current in the associate muscle. The finding that a negative variation accompanies nerve transmission and muscle action seemed directly analogous to the production of induced current.[18] A necessary condition for

[18] Ibid., 300-303, for the direct analogy between the phenomenon of induction as demonstrated by Faraday and his own experiments on the negative variation; and *UTE 2* (1849), 388-389, for analogy between Faraday's "electrotonic state" and the phenomena of electrotonus associated with the nerve.

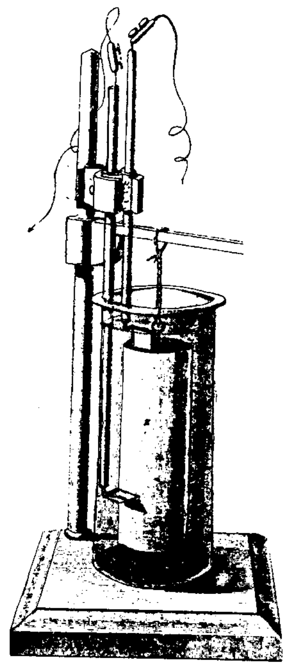

FIG. 9 DuBois-Reymond's physical model for the muscle current. A hollow copper cylinder, galvanized on the outside, hangs in a beaker containing an electrolytic fluid. Leads from a galvanometer run to the outside and to the interior of the cylinder. DuBois-Reymond , *UTE, 1* (1848).

the complete applicability of this model to neurophysiology seemed to be a *preexistent* current in the nerve and muscle. In order to render the analogy complete, DuBois-Reymond focused on the muscle and nerve currents a central phenomena. The models adapted from Faraday's work served as schema for selecting the principal phenomena and ordering their relative importance. Having committed himself to the centrality of the nerve and muscle currents, DuBois-Reymond dogmatically defended them throughout the rest of his career.

But it was not just concepts from electricity and magnetism, such as induction or the electrotonic state, that were important for DuBois-Reymond's enterprise. Equally important were the experimental arrangements and instruments through which Faraday demonstrated the phenomena of induction. They provided DuBois-

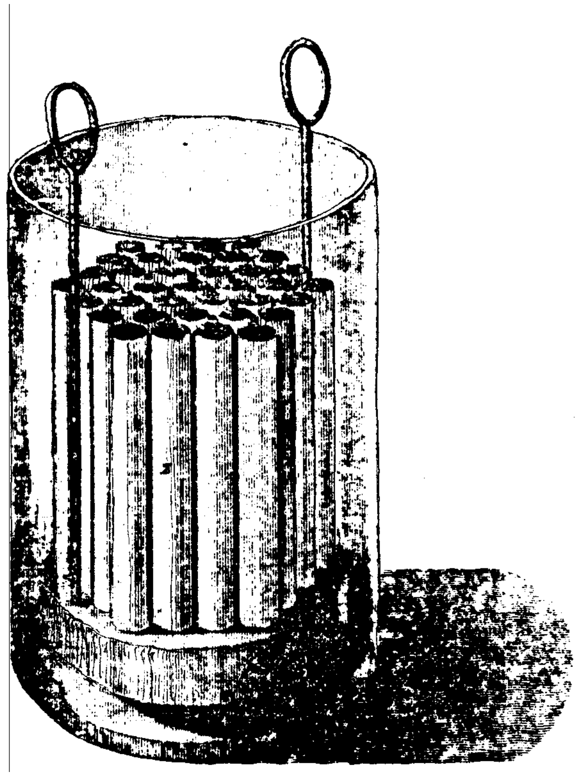

FIG. 10 DuBois-Reymond 's physical model of the muscle. Muscles consist of bundles of electromotive cells similar in structure to parallel cylinders submerged in an electrolyte. Each cylinder has a zinc exterior and a copper interior. DuBois-Reymond, *UTE, 1* (1848).

Reymond with a rich source of models he needed for interpreting all aspects of the action of nerves and muscles in terms of electromagnetic theory. A case in point is the apparatus used in detecting the negative variation illustrated in figure 4. The relationship between the primary coil P and the secondary coil S was a model for conceptualizing the mechanical connection between the muscle current and its negative variation. Just as Faraday had postulated that the secondary is put in an electrotonic condition when current flows through the primary and that any fluctuation of the current induces a flow of current in the secondary, so DuBois-Reymond imagined the mechanical connection betty en the muscle (or nerve) current and negative variation. He went so r as to depict this analogy in figure 5 by designating the elements of is physiological rheoscope as the primary P and secondary S.

Having constructed models for selecting and interpreting the phenomena in terms of electromagnetic theory, DuBois-Reymond turned to the construction of theoretical models depicting underlying molecular mechanisms for generating the phenomena and their causal interrelationships. The work of Ampère was central to this aspect of DuBois-Reymond 's efforts. In their *Exposé des nouvelles découvertes sur l'électricité et le magnétisme*, published in 1822, Ampère and Babinet had depicted magnets as composed of serially arranged Bipolar magnetic molecules.[19] The phenomena connected with the electrotonic state-in particular the direction of the current flow in the extrapolar regions of the nerve-suggested an analogy to the alignment of dipolar molecule postulated by Ampere and Babinet's discussion of magnets. DuBois-Reymond was also encouraged in forming this assumption by Faraday's work on electrolysis, which pointed to a model of electrical dipoles as the mechanical basis for the electrotonic state.[20]

On the strength the of these analogies, DuBois-Reymond postulated the existence of electrically charged dipolar molecules in muscles and nerves. In the normal state of the muscle, these dipolar molecules combined into "peripolar" electromotive molecules negatively charged on each of their poles and positively charged on their equatorial band.[21] DuBois-Reymond assumed further that these molecules would be aligned in the element of muscle or nerve fiber so that all of the negative poles were situated along the cross section while the positive equatorial sector faced the lateral surface of the fiber (figure 11). Bathed in the surrounding electrolytic solution of the nerve and muscle, these molecules would give rise to the constant current manifested as the muscle or nerve current. In order to accommodate the finding that muscles terminated by tendons frequently either did not exhibit a resting current or at best a very weak one, DuBois-Reymond supposed that at its natural termination a muscle would be bound by a layer of dipolar molecules. These would generate a compensating current.

In order to account for the phenomena connected with electrotonus, DuBois-Reymond assumed that the applied current initiates a process of electrolysis in the nerve, leading to the breakdown of the peripolar molecules into dipolar molecules.[22] The dipolar molecules turn under the current until they align polewise. In this process, initiated by closing he circuit, a momentary flow of current occurs. The molecular arrangement is the physical basis of the electrotonic state in the nerve. When the electrotonic state alters, the molecules return to their normal arrangement in the resting state. The rotation of the

[19] *UTE, 2* (1849), 678-683
[20] Ibid., 388.
[21] *UTE, 1* (1848), 678-683.
[22] *UTE 2* (1849), 320-329.

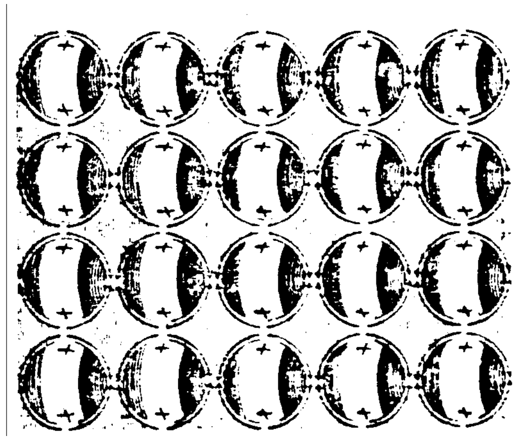

FIG. 11 DuBois-Reymond 's peripolar molecules. Each nerve fiber or muscle bundle consists of collections of molecules having a negative charge at the poles and a positive band of charge along the equator. Each peripolar molecule consists of several dipolar molecules. Under certain circumstances, such as during electrotonus, the peripolar molecules dissociate in a process of electrolysis into dipolar molecules. DuBois-Reymond , *UTE, 1* (1848).

dipoles again leads to a momentary flow of current.

This model was quickly found to be wanting. While it accounted for the direction of current flow observed in the extrapolar regions, it was completely inadequate for dealing with the phenomena associated with the region between the two stimulating electrodes. The model assumes a constant condition in the intrapolar region. Eduard Pflüger soon demonstrated, however, that the nerve is polarized between the stimulating electrodes, passing from negative near the cathode to a positive region near the anode. Furthermore, the model did not provide a mechanism for understanding the differences in excitability associated with the anode and cathode, which came to be the focus of subsequent models.

3. CHEMICAL APPROACHES

Considering the vehemence, if not to say rancor, with which DuBois-Reymond defended his views, it is not surprising that he met with considerable opposition. The aspect of his theories that drew most criticism was the attempted reduction of the entirety of nerve and muscle action to electromotive forces. An alternative proposed by one of DuBois-Reymond 's students, Ludimar Hermann (for which DuBois-Reymond never forgave the ungrateful wretch!), was adopted widely by physiologists such as Pflüger and Julius Bernstein.

Hermann's objections to DuBois-Reymond's electromolecular theory derive from a criticism raised by Helmholtz in 1852.[23]

[23] Hermann Helmholtz, "Die Resultate der neueren Forschungen über thierische Elektricität," *Allgemeine Monatsschrift fur Wissenschaft and Literatur,* 1852, reprinted in Helmholtz, *Wissenschaftliche Abhandlungen,* 2 (Leipzig, 1883), 888-923.

Helmholtz pointed out that it was impossible to tell whether a constant current is actually present in *uninjured* muscle bundles as DuBois-Reymonld postulated. He implied that DuBois-Reymond 's muscle current might be the product of injury. Hermann found experimental evidence that confirmed Helmholtz's suspicion and undermined DuBois-Reymond's fundamental assumption of a permanent current flowing in uninjured nerves and muscles. This assumption, we know, enabled him to build upon Faraday's concepts of electromagnetic induction and the electrotonic state to construct models for interpreting nerve-muscle interaction and muscle contraction.

Hermann's finding had important implications for those insistent upon pursuing la strictly mechanistic electromolecular account. It meant that something like an electrical *potential* had to be assumed in place of DuBois-Reymond's resting current. This in turn implied that muscles and nerves had to be conceived more like capacitors than like batteries and that mechanisms had to be proposed both for generating the potential as well as for discharging it. Finally, if nerves and muscles were to be conceived of as collections of capacitors, some mechanism had', to be proposed for transmitting signals from one "cell" to another.

The speed of transmission also raised problems for DuBois-Reymond's theory. In his critique, Helmholtz had favored the idea that electrically charged particles caused the nerve and muscle currents. Neglecting the shape of the molecules- Helmholtz thought that dipolarity aid peripolarity were too speculative to worry about- Helmholtz observed that the rapidity with which nerve segments respond to reversals in polarity of an applied current in electrotonus spoke strongly in favor of an electrical rather than a chemical basis of transmission. He noted that DuBois-Reymond 's muscle current could be referred to alternating layers of acidic and alkaline substances housed within a chemically neutral sheath, but it was difficult to imagine how such a mechanism could restore itself quickly enough to account for the observed reversals in polarity. Yet, while he favored an electrical theory for the transmission of nerve impulses and muscle excitation, Helmholtz thought that the underlying mechanism for nerve and muscle action would probably be chemical in character. In support of a chemical-molecular approach, Helmholtz drew upon his pioneering investigation done in 1850 with the myograph, which established that the speed of transmission in nerves is considerably less than that of 'electrical transmission in a normal conductor.[24]

[24] See Helmholtz, "Messungen über den zeitlichen Verlauf der Zuckung animalischer Muskeln and die Fortpflanzungsgeschwindigkeit der Reizung in den Nerven," reprinted in *Wissenschaftliche Abhandlung* (ref. 23), 764-843.

Concerning how the activity of nerves and muscles could be linked to accompanying changes of an electrochemical nature, Helmholtz feigned no hypotheses.

For Herman, these formidable problems implied that the entire approach take by DuBois-Reymond ought to be abandoned. In place of such a physical reductionist account-by which reduction to *mechanics* was generally understood-Hermann proposed what he called a "physiological model" of nerve and muscle action, the theoretical components of which were deeply dependent on models of biochemical pathways.

Central to Hermann's model was incorporation of what was then known about t e chemical composition of nerves and muscles in resting and in active states as well as after all signs of excitability had disappeared in rigor mortis.[25] Through a variety of experiments Hermann had established that in rigor mortis a coagulation of muscle components occurs, leading to the shortening and stiffening of the muscle fibers. He had established that the central components in this process seemed to be glycogen, myosin, phosphoglyceric acid, lactic acid, and carbon dioxide.[26] By comparing the muscle fiber components at various stages during the onset of rigor mortis, Hermann concluded that there was a continual increase in the formation of myosin, along with a corresponding consumption of glycogen and an increase in the formation of lactic acid and carbon dioxide.

Since analysis of the active muscle revealed the same substances, Hermann too the processes leading to rigor mortis as analogous to the pathway leading to the release of energy in the active muscle. He envisioned a cyclical biochemical pathway in which, during the restitutive or synthetic phase, glycogen and oxygen were combined with some-as yet unknown-protein into what he termed an "energy generating" *[krafterzeugende]* or *"inogenic"* substance.[27] He supposed that this substance as highly unstable. Hermann did not detail what initiated the dissociative process, but it is clear from the role that this "inogenic" substance played in other contexts that any stimulus, such as an electrical shock, which disturbed the delicate state of equilibrium would et things going. During muscle action, the "inogenic"

[25] Ludimar Hermann, *Untersuchungen über den Stoffwechsel der Muskeln ausgehend von des Gaswechsel derselben* (Berlin, 1867). The results reported in this book are extended and summarized in the revised third edition of Hermann's textbook, *Grundriss der Physiologie des Menschen* (Berlin, 1870), esp. 233-239. The *Grundriss* was published first in 1863 .
[26] Hermann, *Untersuchungen* (ref. 25).
[27] Hermann, *Grundriss* (1870, ref. 25), 236-237. Hermann first proposed the cyclical pathway in *Utersuchungen* (ref. 25), -78-83. There, he made use of Kekule's structural models for molecules to explain energy release and storage (pp. 69-70).

substance broke down into phosphoglyceric acid, lactic acid, carbon dioxide, and, most importantly, myosin. The release of energy accompanied this process.

Breakdown products, such as carbon dioxide and lactic acid, were removed by the blood. (Hermann did not discuss further the fate of phosphoglyceric acid, and was unaware that it is a step in the glycogenic cycle leading to the formation of pyruvic and lactic acid.) The myosin, however, remained as a "ferment" for the restitution of the inogenic substance in the oxydative, synthetic phase of the pathway.[28] Building on Max Traube's work on hemoglobin, Hermann speculated that myosin would unite with oxygen and an unknown nitrogen-free atom complex to produce the "inogenic" substance.[29]

Hermann used this cyclical model of energy release to account for the electrical effects described by DuBois-Reymond . Based on the analogy between the onset of rigor mortis and energy release in the active muscle, Hermann described the biochemical pathway leading to energy release in the active muscle as a transient alteration in the muscle analogous to a "momentary rigor mortis" *[Erstarrung]*.[30] Noting that dead tissue always exhibits electronegativity with respect to intact tissues, Hermann argued that DuBois-Reymond's "muscle current" resulted from injuring the muscle, thereby initiating the onset of chemical alterations similar to those leading to rigor mortis. The biochemical model a' the basis of the alteration theory described above would suggest, Hermann argued, that a sectioned muscle would exhibit a flow of current from the intact surface to the damaged, and hence dying, cross section.

Building on the alteration theory, Hermann proposed that when a nerve is stimulated, a region at the point of stimulus begins to undergo chemical alteration. This alteration renders the region electronegative relative to regions immediately in contact with it. Positive charge from their neighboring sectors flows to the altered sector, and initiates the chemical mechanisms controlling alteration in the adjacent region. This adjacent region in turn becomes electronegative with respect to its adjacent sector, and so on. Sectors that have been altered remain in a refractory state until biochemical mechanisms within the nerve restore them to normal. In this way a stimulus initiated at a point) mid-way along the nerve will travel outward to both ends of the nerve (figure 12); similarly, a stimulus applied at one end will be transmitted along the entire length of the nerve.

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[28] Hermann, *Grundriss* (1870, ref. 25), 237-238.
[29] Ibid., 239.
[30] Ibid., 255-257.

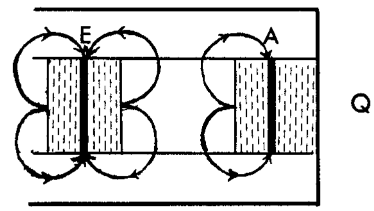

FIG. 12 Nerve transmission and communication of action wave in muscles according to Hermann's alteration theory. In a stimulated region *E* of the nerve or muscle, chemical changes take place analogous to those in rigor mortis; these render *E* temporarily electronegative with respect to neighboring nerve or muscle's tissue. The flow of current from the neighboring regions makes them electronegative to regions more remote, and so on. The initial stimulus rapidly travels down the length of the nerve or muscle fiber to successive positions, such as *A* . This is my rendering of a similar diagram in Biedermann *Elektrophysiologie* (1895), (ref. *39).*

Hermann's physiological model could account for all the data in DuBois-Reymond's ponderous electrophysical researches. It was also in accord with new findings, such as Bernstein's demonstration in 1866 that as the "action wave" proceeds along the muscle, every point within the active sector is electronegative with respect to every segment of muscle at rest. (In Hermann's view, active segments are undergoing "alteration" and exhibit the expected electronegativity.) Moreover, since transmission depended upon time-consuming chemical transformations, the stimulus-signal would be much slower than a current in a metal, and Hermann's model could accommodate Helmholtz's findings on the time required for stimulus transmission in nerves.

Although Hermann employed current flow in his model of stimulus transmission, electrical phenomena served only as one ingredient in his general explanatory model, which derived from the pathbreaking work then being done in physiological chemistry.[31] The phenomena of

[31] At the outset of his career DuBois-Reymond regarded the underlying processes as chemical, and he never doubted that the ultimate explanatory framework in neurophysiology would be electrochemical. He wrote Carl Ludwig on 22 Oct 1847: "In muscles, and most probably) also in nerves, the known processes are not merely of a mechanical nature; chemical transformations take part in the process. However, since all chemistry has so far been nothing but shopkeepers' bookkeeping, not even the simplest process having been worked out with analytical understanding, and since the chemical processes here are not know exactly and will most probably be of a very complex nature, you can surely see what little hope there is of arriving at a clear concept of the molecular mechanism of contraction." Estelle DuBois-Reymond and Paul Diepgen, eds., *Zwei grosse Naturforschfer des 19. Jahrhunderts. Ein Briefwechsel zwischen Emil DuBoisReymond and Karl Ludwig* (Leipzig, 1927), tr. Sabine L. Ayed and Paul Cranefield (Baltimore: Johns Hopkins, 1982), 4.

So-called animal electricity in Hermann's view were all artifacts of more fundamental physiological processes. They could be detected and followed by electrical recording devices, but they were not merely electrical phenomena. For Hermann, the most important factor was contact between layers of tissues along the cross-section of nerves or muscles, which though adjacent, were in chemically different states, corresponding o different molecular arrangements, and so capable of liberating chemical energy. To Bernstein's objection that the "alteration theory" was merely a convenient description of the known facts that relied on unspecifiable chemical transformations, Hermann responded that DuBois-Reymond's "electromotive theory" could claim no more. Furthermore, Hermann objected to the number of independent assumptions in DuBois-Reymond 's theory, such as the need for a layer of dipolar "parelectric" molecules at the ends of muscle fibers that do not exhibit a muscle current. In support of his own model, Herman argued that it could be more readily accommodated to the physiological mechanisms being developed in other areas, such as. Traube's model for oxygen transport via the hemoglobin molecule or Haidenhain's model for secretion.

A major area of investigation during the 1860s was phenomena connected with the electrotonic state, the condition of the nerve (or muscle) when current is externally applied. Exploration of these phenomena were made possible by nonpolarizing electrodes introduced by DuBois-Reymond in 1859, most important and long-lasting of his contributions to neurophysiological instrumentation.[32] Nonpolarizing electrodes were constructed from zinc strips amalgamated with a thin coating of zinc sulfate and dipped into a tube containing a concentrated solution of zinc sulfate. The tip of the electrode was formed from porous modeling clay soaked in a 0.7% saline solution (figure 13). These electrodes were nonpolarizing because they employed a chemical reaction reversible with respect to Zn^{++}.[33] These new electrodes voided the generation of potentials due to the contact of polarizable metals, which had been an especially difficult problem in previous investigations of nerve transmission.

Eduard Pflüger, working with the new nonpolarizing electrodes in DuBois-Reymond's laboratory in 1859, was the first to make

[32] Emil DuBois-Reymond, "Über gleichartige and nicht polarisierbarende Elektroden," Akademie dei Wissenschaften, Berlin, *Monatsberichte*, 1859, 443-488.

[33] Electronic conduction in the metal ($Zn^{++} + 2e^{-}$ < -> Zn) is mediated by the coating of zinc sulfate, the solubility of which is low, providing a store of Zn^{++} and SO_4^{-2} ions-which in turn participate in the ionic current involving the exchange of SO_4^{-2} -between the strips and the concentrate solution of zinc sulfate.

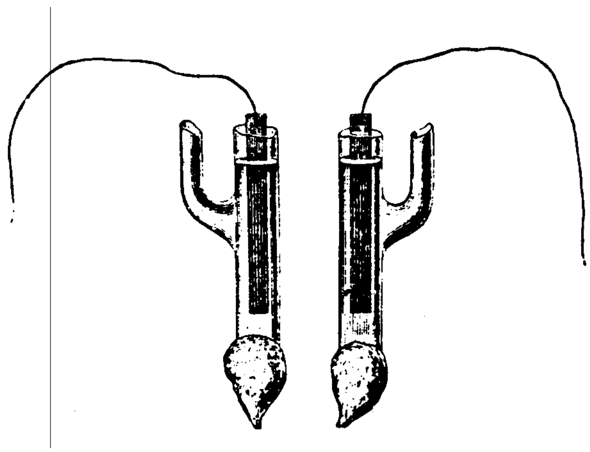

FIG. 13 DuBois-Reymond's nonpolarizing electrodes. Zinc strips coated with a layer of zinc sulfate dip into a concentrated solution of zinc sulfate. The tips of the glas tubes containing the electrodes and electrolytes are made of modeling clay Soaked in saline solution. Bernstein, *Lehrbuch* (1900) (ref. 40).

important new findings about electrotonus. They inspired Hermann to design a fruitful model. DuBois-Reymond had demonstrated that in the extrapolar regions of a nerve through which a constant current flows, the nerve current has the same direction as the constant current. He had demostrated further that the negative variation sums algebraically with the electrotonic current. Before 1859, when he introduced his nonpolarizing electrodes, DuBois-Reymond was not able to investigate the behavior of the nerve in the intrapolar region with the means at his disposal. His model of the serial arrangement of dipolar electromotive molecules, however, suggested that the nerve should behave identically in the intra- and extrapolar regions. Pflüger disconfirmed he suggestion. Through careful measurements using DuBois-Reymond's improved electrodes, he showed that the nerve is polarized between the stimulating electrodes, positively near the anode and negatively near the cathode (figure 14). With increased current the null point approached the cathode .[34] Pflüger also cataloged the strength of muscle contractions as a function of the electrotonic current and its direction, and whether the contraction occurred during the opening or closing of the circuit.

Hermann explained all of these effects in terms of a *Kernleiter* or cable mode1.[35] He argued that during electrotonus the nerve behaves like a tube filled with an electrolytic solution through the center of

[34] Eduard Pflüger, *Untersuchung über die Physiologie des Electrotonus* (Berlin, 1859), 247 ff.
[35] Hermann in *Pflüger'sArchiv,* 5 (1872), 223-275, on 270.

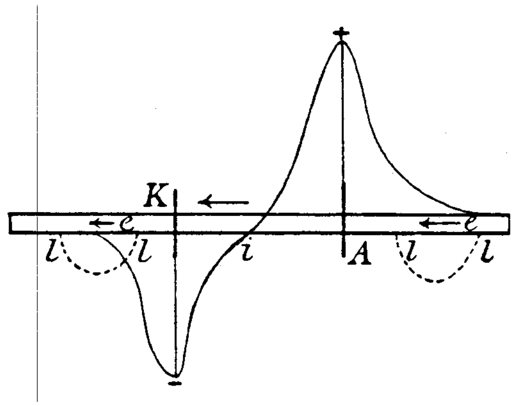

FIG. 14 Behavior of nerve in electrotonus as measured by Pflüger. The current *e* flows from *A* to *K.* Exploratory electrodes *ll* disclose that the region around *A* is positive, at *i* neutral, around *K* negative. As *e* increases, *i* moves toward *K.* **Pflüger**, *Untersuchung* (1859) (ref. 34).

which a conducting wire passes. When an electric potential is applied between two points on the wall of the tube, the region surrounding the electrodes becomes polarized; according to Hermann, positively charged electrolyte accumulates around the anode and negatively charged electrolyte around the cathode (figure 15). Hermann constructed a

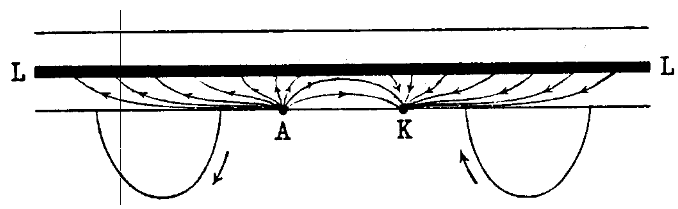

FIG. 15 Hermann's cable model. A conducting wire, LL, bathes in an electrolytic fluid. Current is applied at A, conducted by the fluid to the wire, through the wire, and via the fluid to K. The effect is attenuated by increasing the distance between A and K. The loops on the exterior of the cable indicate the direction of current a galvanometer would register if placed in a metallic circuit coincident with the loops. Points in or on the able right of K are progressively positive with respect to K, and points left of A are progressively negative with respect to A. Hermann in *Pfüger's Archiv, 5* (1872) (ref. 35).

physical model consisting of a glass tube corked at both ends with a platinum wire running down the center (figure 16). The tube was filled with a dilute solution of sulfuric acid and contained capillary junctions placed at regular intervals with recording electrodes attached so that he behavior of the *Kernleiter* could be investigated at different points. The model conformed to the phenomena exactly. More importantly, it harmonized with the physiological model Hermann had developed to describe nerve and muscle action, for a direct analogy could b e drawn between the electrolytic solution of the *Kernleiter* and the biochemical solutions bathing nerve and muscle.

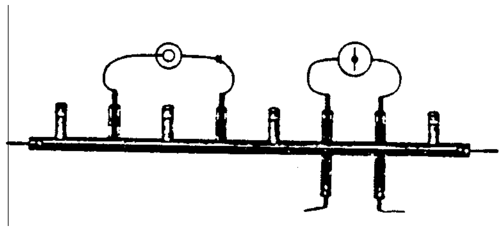

FIG. 16 Hermann's apparatus for modeling the phenomena of electrotonus. A silver conducting wire is immersed in a dilute solution of sulfuric acid and placed in a glas tube corked at both ends. Capillary junctions are placed so that recording lectrodes and stimulating electrodes can be variously positioned in order to explore effects in both intrapolar regions and extrapolar regions. Biedermann *Elektrophysiologie* (1895) (ref. 39).

Pflüger also designed a model of the essential features of the physiological-chemical account of nerve action.[36] In contrast to the other types of models we have encountered, Pflüger's is a *formal analogy*. Bernstein frequently described this formal analogy in his accounts of the membrane theory, and I believe that it assisted greatly in the development of the theory of nerve and muscle action. Pflüger emphasized tat his model was not a description of the underlying causal process s in nerve action, but a representation of the essential qualitative features to be incorporated into a theoretical account. The model he proposed was the antithesis of refinement.

Pflüger began from the assumption that a general theory of nerve and muscle ac ion had to incorporate Helmholtz's work on the conservation of energy.[37] He also noted that, given the low value obtained by Helmholtz or the transmission speed of nerve stimuli, the underlying mechanisms in a theoretical model would have to be chemicalmolecular and not electric.[38] Pflüger assumed that, consistent with the first law of thermodynamics, everywhere in nature where potential energy is store an opposing force must be assumed for equilibrium. The object of theory construction would be to find chemical-molecular interpretations for the forces responsible for potential build-up and for the forces maintaining equilibrium in the rest state.

Pflüger's formal analogy consisted of an L-shaped cylinder with a piston K attached at one end by a spring S (figure 17); 11 is a diagonal slit in the cylinder wall covered by K when the cylinder was full of water. The water represented the stored potential energy in the nerve,

[36] Pflüger, *Untersuchung* (ref. 34), 480-481.
[37] Ibid., vii.
[38] Ibid., 492.

the column A being the portion available for conversion into kinetic energy. The spring represented the molecular forces—whatever they

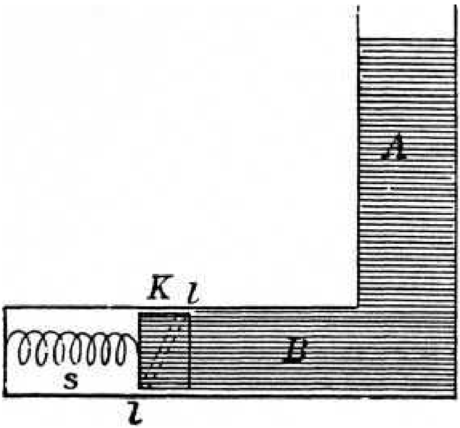

FIG. 17 Pfluger's formal analogy for depicting the principles of nerve and muscle mechanics. The spring *s* and piston *K* represent the molecular forces opposing the "potential energy" stored in the nerve, represented here by hydrostatic pressure in the tube *AB*. Pfluger, *Untersuchung* (1859) (ref. 34).

might be—maintaining equilibrium. Any addition of water to the cylinder (corresponding to a stimulus of some sort) would compress the spring and expose the slit, allowing water to run out until equilibrium was restored. To improve the analogy to the kinetic energy developed during muscle contraction, the shape of the slit could be modified so that water ran out rapidly at first and then more slowly; and, to ape the basal metabolism of the resting nerve (or muscle) segment, a small trickle of water could be constantly added to the cylinder to produce a compensating flow through the slit.

Pfluger's model summarized the considerable body of phenomenological laws connected with nerve action that had piled up since DuBois-Reymond's initial investigations.[39] One such law, found by Pfluger, stated that the excitability of a polarized nerve is increased (i.e., the nerve becomes more capable of initiating a muscle contraction or registering a "spike" or negative variation) at the cathode when the circuit of the polarizing current is closed, and increased at the anode when the circuit is broken.

The closing of the polarizing circuit increases the potential energy of the system. Since contractions originate from the cathode when the circuit is closed, the equilibrium must be disrupted at the cathode.

39. Wilhelm Biedermann, *Elektrophysiologie, 1* (Jena, 1895), 716-721.

Correspondingly, since no contractions are initiated at the anode until the circuit is opened, the polarizing current must increase the molecular forces in the nerve maintaining equilibrium there. Pfluger represented these conditions in the model by a momentary decrease in the elasticity of the spring when the circuit is closed; the consequent flow through the slit corresponds to the potential released from the cathode. The spring's elasticity may be so altered by an intense polarizing current that the piston can be pushed back far enough to allow multiple nerve firings from the cathode.

The phenomena connected with the anode also followed from alterations in the spring constant. While the polarizing current runs, the elasticity of the spring will increase, perhaps even beyond its strength in the resting state. If the spring is long enough, the piston may press the column of water well beyond the slit. When the polarizing current is turned off, the increase in the molecular forces within the nerve that opposed the conversion of the increased potential energy of the system in electrotonus into kinetic energy will be removed, the piston will be depressed well beyond the slit as a result of the release of the potential accumulated during electrotonus, and a nerve firing will be initiated from the anode.

In Bernstein's hands, this model served as a heuristic device for clarifying the structural characteristics to be incorporated in a future model of the underlying mechanism giving rise to the phenomena of nerve excitation during electrotonus.[40] A molecular mechanism had to be imagined that would lead on the one hand to a *decrease* in the potential (by altering the spring constant) at the cathode and at the same time produce an *increase* in the potential at the anode. In his presentation of the membrane concept in 1912, Bernstein took pains to show that his ion model satisfied the conditions depicted in Pfluger's formal analogy of a dynamic equilibrium between opposing mechanisms for generating and releasing potential energy in nerves and muscles.[41]

4. BERNSTEIN'S EARLY WORK: REFINEMENTS IN INSTRUMENTATION

Like his teacher DuBois-Reymond, Bernstein concentrated much of his early efforts on devising experiments to demonstrate the

40. Bernstein, ***Lehrbuch der Physiologie des thierischen Organismus im Speiellen des Menschen*** **(Stuttgart, 1900), 398-406 (1st ed., 1894).**

41. Bernstein, ***Elektrobiologie. Die Lehre von den elektrischen Vorgangen im Organismus auf moderner Grundlage dargestellt*** **(Braunschweig, 1912), 138-141.**

electrical nature of nervous transmission and muscular contraction. Essential to establishing the causal role of bioelectric currents in these processes was a determination of the behavior of the negative variation at sucessive positions along the nerve, as well as the role of the variation in the onset and transmission of the contraction wave in the muscle. To this end, much in the spirit of DuBois-Reymond, Bernstein improved the measurement and recording devices used in neurophysiology. His most important contribution along these lines was the development of the differential rheotome.[42] The instrument (figure 18) consisted of a rotating wheel R with contact points p1 and p_2, which dip into quicksilver baths Q once every rotation, thereby closing the circuit containing the recording electrodes 1 and q and the multiplicator M. At the opposite end of the wheel is another contact point, p, which closes the circuit with the stimulating electrodes and induction apparatus, aRpdPrSLrr.

The aspect of the device that made it useful for probing electrophysiological events was its contact d, located on the outer wheel. It was made adjustable. By moving d radially, Bernstein could vary the time between the stimulus and the response at the recording electrodes. This made it possible to investigate the change in the negative variation at the point 1 during different phases of the action wave, including its maximum and minimum. Bernstein's results for several such experiments over time intervals ss appears in figure 19.

From such experiments Bernstein concluded that the negative variations never exceed the "muscle current" or resting potential of the muscle. The maximum of the negative variation in figure 19 never sinks below the abscissa oo, the value of current registered before the onset of the contraction wave. By varying the strength of the stimulus current, Bernstein could show that with weak stimulation the graph of the negative variation might not reach the abscissa line; but even with extremely intense stimulation, the curve never dipped below the value for the abscissa.[43]

The differential rheotome could also be used the check the nature of the transmission characteristics of the negative variation. The graph generated by the stimulus wave as it passes two different muscle points, l_1 and 1_2, appear in figure 20; l_1 is electronegative with respect to l_2. When the stimulus wave passes to position 2, 1_2 becomes electronegative with respect to l_1 (the arrows in the diagram indicate the direction of current flow). The curves of the changes in the negative variation are different at points 1 and 2, however, because by the time

42. Julius Bernstein, *Untersuchungen uber den Erregungsvorgang in Nerven und Muskeln* (Heidelberg, 1871).
43. Ibid., 26-28.

the wave has reached 2, the muscle has begun its restitutive phase. One inference from these findings was that if recording electrodes are placed near one another, the graph of the "negativity wave" should be formed by combining the two curves, resulting in a double-phased action curve.

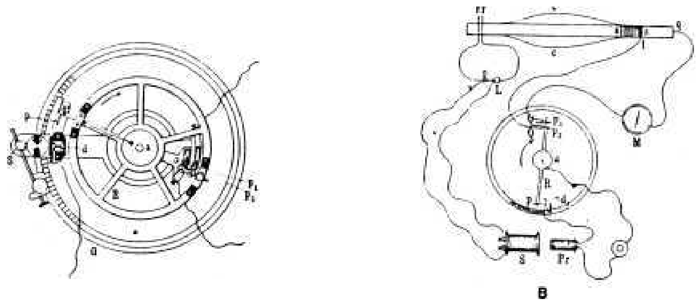

FIG. 18 **Bernstein's rheotome.** The rheotome (viewed from above in 18 A) consisted of a horizontal wheel R, which rotates about a central axis a. P, a steel needle pointing downward at about 45 degrees, is connected by a wire to a and thence to a battery and primary coil Pr (18 B). The primary circuit closes once every revolution of R, when P makes contact with a wire, d, on a second horizontal wheel, G, below R (18 A). The position of the contact d could be adjusted by means of a micrometer, S, attached to G (18 A). When the primary coil is placed within the secondary coil S and the switch L is closed, an induced current flows to the stimulating electrodes rr once every revolution of R (18 B). Directly opposite P is a second contact, consisting of two copper needles p1p2 joined by a wire and isolated from R. Once every revolution p_1p_2 briefly graze the surface of the mercury baths Q, thereby closing the circuit lQP_2P_1Mq, including the galvanometer M and the nerve (or muscle) segment lq. While the recording circuit is closed, the galvanometer registers the constant "resting current" of the nerve (or muscle). Owing to the sluggishness of the galvanometer, a rotation of R of 5 or 10 times a minute gave pulses that registered as a constant current. The interval during which the recording circuit was closed depended on the time the p_1p_2 remained in contact with the mercury baths. In order to be able to vary this interval, Bernstein attached the mercury baths Q to adjustable brass arms. By moving the arms toward or away from one another, the contact interval could be lengthened or shortened. In order to investigate the electrical changes in the nerve (or muscle) associated with the stimulus, the opening and closing of the recording circuits had to synchronize with the opening and closing of the stimulating circuit. Figure 18 B illustrates the situation in which contact between p and d initiates a stimulus current at the moment p_1p_2 leaves the mercury baths. The recording circuit is then broken, and stays open for between 0.10 and 0.20 sec. During this period the negative variation due to the arrival and passage of a stimulus at 1 would have run its course. To catch the arrival of the stimulus at 1 and the beginning of the negative variation, the contact point d must be moved to some point, such as d_1, far enough along G so that the recording circuit remains closed for a period of time (corresponding to the angle of rotation d_1ad) sufficient for the stimulus to travel the length of the nerve (or muscle) and arrive at 1. As d is moved successively beyond d_1, the successive values of the negative variation, including its maximum and return to zero, will register on the galvanometer. Bernstein *Untersuchungen* (1871) (ref. 42).

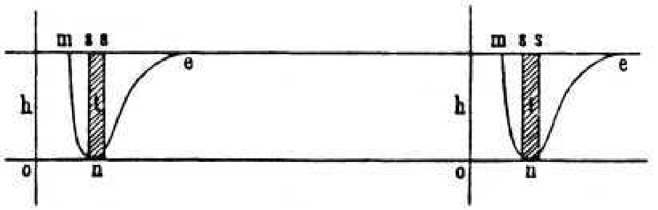

FIG. **19** **Temporal course of the negative variation.** The horizontal line oo represents time, the ordinates represent the muscle current as measured by the galvanometer. The resting current is given by h. The points o represent two successive rotations of the rheotome, and hence two successive stimuli initiated at rr in figure 18. The ss represents the time the galvanometer circuit is closed during one rotation of the rheotome wheel. The rectangle t represents the total current passed during ss. By making ss as small as possible, the curve of the negative variation can be approximated. In the diagram on represents the time it takes the stimulus wave to travel from rr to 1. mne represents the negative variation of the muscle current as the stimulus passes 1. Bernstein *Untersuchungen* (1871) (ref. 42).

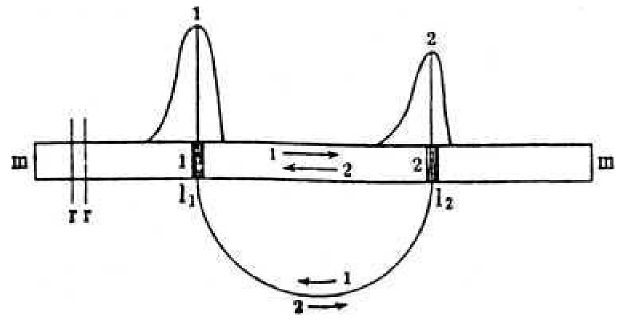

FIG. 20 **Transmission of the stimulus wave as revealed by the differential rheotome.** The muscle m is stimulated from rr. When the stimulus wave reaches 1, the segment of muscle there is rendered electronegative relative to segment 2; the direction of current flow detected by the galvanometer is represented by the arrow 1, showing current flowing from l_2 to l_1. When the stimulus wave reaches 2, the surrounding area becomes negative relative to segment 1. Segment 1 does not fully regenerate its potential by the time the stimulus wave reaches segment 2. Bernstein *Untersuchungen* (1871) (ref. 42).

These and related experiments led Bernstein to important phenomenological laws relating to nerve and muscle action. By comparison with Helmholtz's results on the speed of the contraction wave using the myograph, Bernstein could show that the negative variation is transmitted at the same velocity as the contraction wave.[44] He could show, furthermore, that the negative variation occurs in the period of latent stimulation of the muscle, and hence, that it *precedes* the onset of the contraction wave.[45] This was a major contribution

44. Ibid, 22-23.
45. Ibid., and *Lehrbuch* (ref. 40), 319.

toward establishing the claims of DuBois-Reymond concerning the centrality of the negative variation and the electrical nature of the events.

Bernstein drew further consequences from his experimental researches that bore on the construction of a theory of the underlying mechanisms. His experiments showed that in each muscle segment the negative variation has almost entirely run its course *before* a noticeable contraction begins. This seemed to indicate that the negative variation is a molecular process, and that it prepares the onset of the contraction.[46] These results fit well with Helmholtz's famous measurement of the speed of stimulus transmission and muscular contraction. The results also fit well with experiments on "muscle tone."

In Helmholtz's experiments an electromagnetic tuning fork stimulated a nerve-muscle preparation with intermittent current at known frequency (figure 21). The same device would at the same time be used to send intermittent current to the electromagnets controlling the vibration of the tuning fork in a second instrument, an acoustical interrupter (figure 22). By this arrangement the frequency of the tone generated by the acoustical interrupter was identical to the frequency of the stimulus shocks applied to the nerve. During the resulting tetanus, a stethoscope applied to the muscle made the processes going on in contraction audible. The "muscle tone" and the tone corresponding to the stimulus frequency turned out to be identical. By improving upon Helmholtz's instrument, Bernstein generated muscle tones with frequencies greater than 1000 vibrations per second (figure 23).

In a refinement of this experiment in 1890, Bernstein used a telephone as a means of intercepting and transducing the electrical stimulus wave itself. Once again, there was agreement between the tone of the stimulus wave and the muscle tone. Arguing from the similarity of the tones produced in the tuning fork and in the muscle by the same discontinuous process, Bernstein (following Helmholtz) wanted to draw a direct analogy between the causal processes in both cases. Bernstein argued that the results supported the view that during tetanus, the active processes in the muscle must be *discontinuous* in nature,[47] and they pointed strongly in the direction of an atomic-molecular theory for nerve and muscle action. The identity of frequency between muscle tone and control tone in the acoustical interrupter might be due, he urged, to a one-to-one correspondence between the stimulus pulses and discharge of individual "capacitors" in the muscle.

46. Ibid., 320-321.
47. Ibid., 311-312.

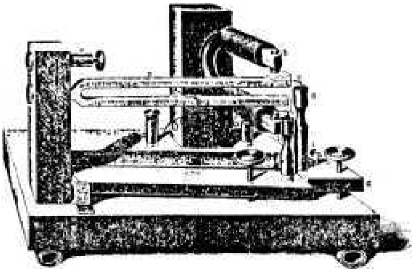

FIG. 21 Helmholtz's electrical tuning fork. A tuning fork a is fixed horizontally between the arms of an electromagnet bb. Platinum wires cc at the end of the tuning fork dip into cups filled with mercury and connected to wires from the clamps at i, which lead to a battery. A third clamp at e is connected to the tuning fork through the insulated housing. When the fork vibrates and a current passes through it from i to e, the current will be broken every time the fork a rises above the mercury and remade when the wire returns to the mercury. The current runs through the electromagnet bb, which becomes magnetic each time the current passes, thereby maintaining the vibrations of the fork. Helmholtz, *Die Lehre von den Tonempfindungen, als Grundlage fur die Theorie der Musik. (Braunschweig, 1863) 198.*

Bernstein's electrochemical theory

Bernstein approached the problem of nerve and muscle action in two phases. Before he hit upon the concept of the membrane potential, he relied on what he called the electrochemical theory of nerve and muscle action.[48] Bernstein described electrical phenomena associated with nerve and muscle action, such as negative variation, as "*signs* [my emphasis] of the chemical energy transformed into heat and work in nerves and muscles."[49]

Bernstein had defended this view in his first major work, *Uber den Erregungsvorgang im Nerven- und Muskelsysteme* of 1871, and he

48. Ibid., 324-340.

49. Bernstein, "Zur Theorie der negativen Schwankung," *Pfluger's Archly, 67* (1897), 349-372, on 370.

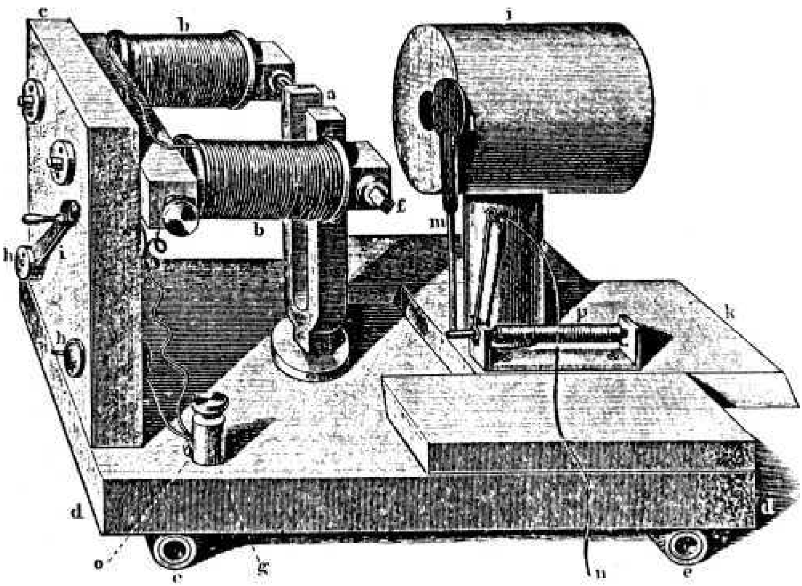

FIG. 22 Helmholtz's acoustical interrupter. A tuning fork a is set in motion by intermittent current produced by a current interrupter. Entering the apparatus at the clamp g, the current pulses flow to electromagnets, bb. Each intermittent current makes the electromagnet temporarily magnetic, and it attracts the prongs of the fork. The vibrations of the fork are amplified by the resonator i. Helmholtz, *Die Lehre von den Tonempfindungen, als Grundlage fur die Theorie der Musik. (Braunschweig, 1863) 196.*

continued to reassert it until three months before the publication of the membrane theory.[50] Although agreeing with their overall approach, Bernstein wanted to go further than Hermann and Pfluger by constructing a model that treated bioelectric currents as one component in a general system of energy transformation in nerves and muscles.

The organization of the muscle tissue in regular parallel fibers suggested to Bernstein that DuBois-Reymond's assumption of a similar structure at the molecular level must be preserved. He drew upon the recent work of structural organic chemists to replace DuBois-Reymond's "peripolar molecules" by atomic complexes of prismatic shape.[51] These atom complexes have a protein able to bind electropositive groups of atoms on the lateral surface and electronegative

50. See Bernstein and A. Tschermak, "Uber die Beziehung der negativen Schwankung zur Arbeitsleistung des Muskels," *Pfluger's Archiv, 89* (1902), 289-331, on 315: "Thus, we are justified in viewing the electricomotive phenomena, particularly the negative variation, as electrical components of particular metabolic processes in muscles."

51. Bernstein, *Lehrbuch* (ref. 40), 325.

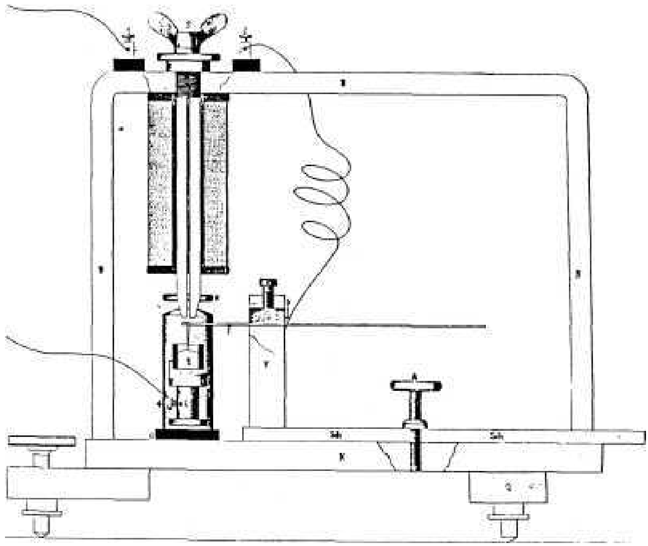

FIG. **23** **Bernstein's acoustical interrupter.** Current from a battery enters the apparatus at terminal 1, passes through the coils of the electromagnet to terminal 2, and continues to a spring F clamped in a housing V. The current passes from the tip of F to a mercury bath q in the cylinder L, and returns to the battery from terminal 4. The housing for the spring can slide along a track Sch, so the length of the spring can be varied. When the circuit is closed, the electromagnet acts, breaking the contact between F and q. The spring is given an initial motion to get things going. The number of vibrations made by the spring is determined by comparing its tone with the tone of a known tuning fork. A spring of 1.5 mm thickness and 17 mm length, for example produces 1380 vibrations per second. Bernstein *Untersuchungen* (1871) (ref. 42).

groups of atoms along the cross section in the direction of the nerve axis (figure 24).

Bernstein did not invent these properties arbitrarily. Rather, drawing on Pfluger's work on the structure of proteins, he assumed that the electropositive atom groups would be molecules capable of oxydation, such as carbohydrates, and that the electronegative atoms would consist of assimilated oxygen. Thus, he reconsituted DuBois-Reymond's peripolar molecules: whereas DuBois-Reymond had assumed permanent microstructural features of nerves and muscles, Bernstein supposed that the structure of the central protein molecules would bind differently charged ions from the surrounding fluids via normal chemical affinities.

Bernstein assumed further that the electrochemical molecules were in a delicate equilibrium that could be easily disrupted by external forces. Arranged in orderly rows and bathed in the electrolytes of the muscle tissue, these molecules would behave exactly like the platinum wire in Hermann's *Kernleiter* when stimulated by an externally

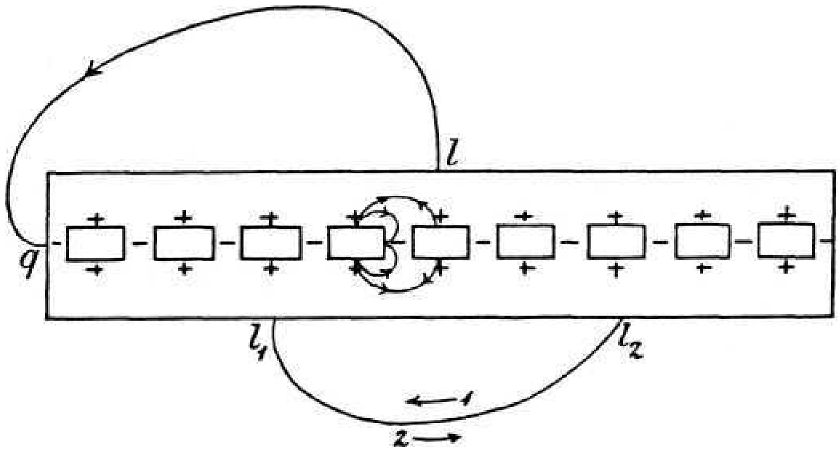

FIG. 24 **Bernstein's electrochemical model.** Nerve and muscle fibers consist of a protein core capable of binding electropositive atom groups, probably carbohydrates, to the surface facing the exterior of the fiber. Electronegative atom groups—assumed to be oxygen—bind to the protein core in the direction of the axis of the fiber. The electrochemical molecules generate a flow of current from *l to q* consistent with the phenomena of muscle and nerve currents. The model can also represent the phenomena connected with the passage of the stimulus wave as depicted by Hermann's alteration theory. As the stimulus moves in the direction l_1l_2, recording electrodes applied at l_1l_2 register a reversal of current flow, represented as 1 and 2 in the diagram. During the refractory period when the electrochemical molecules are being repolarized by the metabolic processes of the nerve or muscle cells, each electrochemical "cell" is electronegative relative to neighboring cells. Bernstein *Lehrbuch (1900) (ref.* 40).

applied current. Similarly, with their positive faces directed toward the exterior nerve or muscle surface and their negative components aligned in the direction of the axis, the "electrochemical molecules" would produce a current through recording electrodes in compliance with DuBois-Reymond's original observations on the muscle current.

Bernstein interpreted the negative variation as the effect of a momentary disruption in the equilibrium state of the electrochemical molecule leading to a "disassimilation" of its oxygen group followed by the rapid "reassimilation" of another oxygen group: "The first part of the negative variation (i.e., its negative phase) depends upon the liberation of active oxygen, the second part (i.e., its positive or regenerative phase) reflects the consumption of the oxygen by the (muscle) contraction___Thus the strength of the negative variation will be a function of the available quantity of active oxygen."[52] Since oxygen-

52. Bernstein (ref. 49), 370.

rich animal tissues were known to be electronegative relative to oxygen-deprived tissues, and since work in muscles was assumed to involve the binding of oxygen and the liberation of heat, Bernstein emphasized transformations involving oxygen as the source of both muscle contraction and its associated electrical phenomena.

Bernstein's electrochemical molecules also offered an interpretation of the molecular processes corresponding to the *Molekularspannungen* and *Molekularhemmungen* depicted in Pfluger's schema for nerve mechanics. Pfluger had pointed to the need for a mechanism, corresponding to the change in the spring constant in his model, which would lead to an increase in the potential in the neighborhood of the anode and a decrease in the potential at the cathode during electrotonus. The "molecular forces" *{Molekularhemmungen)* opposing the transformation of potential into kinetic energy at the anode required by Pfluger's scheme found their interpretation in Bernstein's model in the forces of chemical affinity conditioning the differential accumulation of ions in the vicinity of the anode. Just as in Hermann's *Kernleiter,* so in Bernstein's electrochemical model positive ions accumulate in the area of the anode, negative ions near the cathode. At the cathode, however, the excess negative ions will bind to the positive ions on the surface of the electrochemical molecules forming the nerve fibers; the potential will be reduced, leading to an increased excitability at the cathode. Bernstein speculated that the affinity between the excess negative ions at the cathode and the positive ions on the surface of the molecules would break the weak bonds between the core protein molecule and the oxygen atom complex on the axis. The freeing of the oxygen complex would lead to a release of chemical energy. According to this model, the negative ions in the region of the cathode are mostly freed oxygen ions from the electrochemical molecules.[53]

The situation is reversed at the anode. There positive ions accumulate, leaving the forces binding the (positive) surface ions to the core molecule undisturbed. In fact, the excess positive charge binds additional oxygen ions from the basal metabolic process of the nerve (or muscle) near the anode, thus increasing the potential. When the stimulating circuit is broken, however, "the forces binding the oxygen ions disappear, followed by an internal depolarization of the molecules."[54] Basically, Bernstein assumed that the application of an external current in electrotonus activated and heightened the processes of "assimilation" and "disassimilation" that normally bind oxygen

53. Bernstein, *Lehrbuch* (ref. 40), 403.
54. Ibid., 404-405.

and liberate heat. Accordingly, he predicted that were it possible to construct very sensitive thermometers, the temperature at the anode should be greater than that at the cathode during electrotonus.

Although Bernstein worked with this model for over thirty years, it proved ultimately unsatisfactory. For one, it was qualitative. Also, although it suggested new lines of experimental inquiry (e.g., testing whether the magnitude of the negative variation corresponded to the quantity of oxygen consumed for muscle contraction), they were all highly speculative with little demonstrated grounding in work on the actual biochemical pathways in nerve and muscle action. And many basic assumptions had not been tested: that depolarization of electrochemical molecules could liberate the quantities of energy required, or that oxydation was the source of the electrical currents observed. In a word, Bernstein's model was no more plausible than Hermann's alteration theory. Beginning in mid-1902, Bernstein began to explore the possible relevance of the work of Ostwald and Nernst to electrophysiology. His explorations prompted radical changes in his electrochemical model and elevated the discussion to a new level of precision.[55]

5. THE MEMBRANE THEORY OF BIOELECTRIC POTENTIALS

In 1902 Bernstein set forth his hypothesis on the membrane theory of bioelectric potentials.[56] It inspired a program of research that occupied him and several of his students for a decade. It appeared in final form in Bernstein's last work, *Elektrobiologie,* published in 1912. The new direction in electrophysiology was initiated by the work of Helmholtz and (independently) by Gibbs on the application of thermodynamics to concentration gradients and the generation of liquid-junction or diffusion potentials. Nernst brought their work into a form useful for application to electrolytic solutions separated by a membrane.[57]

Bernstein probably was alerted to the relevance of Nernst's contributions by an analogy between Nernst's derivation of the equations for potential gradients and Pfluger's model for nerve mechanics. Nernst supposed that electrochemical equilibrium was a balance

55. The first person to call attention to the importance of Nernst's work for the theory of bioelectric potentials was Maximilian Oker-Blom, "Tierische Safte und Gewebe in physikalisch-chemischer Bezeihung," *Pfluger's Archiv, 84* (1901), 191-259.

56. Bernstein, "Untersuchungen zur Thermodynamik der bioelektrischen Strome," *Pfluger's Archiv,* 92(1902), 521-562.

57. Nernst, "Elektromotorische Wirksamkeit der Ionen," *Zeitschrift fur physikalische Chemie,* 4(1889), 129-181.

between the electrical work needed to move a small quantity of ions across a boundary in one direction and the osmotic work needed to move the same quantity in the opposite direction. In the derivation of his well-known equation describing the equilibrium condition, Nernst began by considering an ideal gas under a piston in a cylinder, and moved to osmotic pressures by replacing the piston with a semi-permeable membrane and the ideal gas with a dissolved salt. The move gave Bernstein a way to intepret the spring, piston, and water pressure in the old Pfluger model. Furthermore, the ionic theory of solution showed how to unite the features of chemical and electrical models Bernstein had long defended as necessary for interpreting the phenomena of electrophysiology. But even more importantly, the ion theory and the Nernst equation transformed the discussion by making it quantitative.

The form of the Nernst equation relevant to Bernstein's use, and the form in which it appears in most textbooks in physiology today, was:

$$E = \frac{RT}{F} \ln \left[\frac{K_2}{K_1} \right],$$

where E is the potential difference across the membrane, R the universal gas constant, T absolute temperature, F the charge per mole in "Faradays" of the ion under consideration, and K_1, K_2 are different concentrations of the same ion separated by the ion sieve or semi-permeable membrane. One feature of the equation alerted Bernstein to its potential significance in his own field: the electromotive force varies directly with the absolute temperature. Suddenly, one of the well-known phenomenological laws of nerve and muscle physiology took on a completely different light. Hermann had demonstrated that the electromotive force exhibited by nerves and muscles increases with increasing temperature. Although he had not attempted to provide a quantitative relationship, Hermann argued that its qualitative features damaged DuBois-Reymond's theory. He assumed that if DuBois-Reymond's peripolar molecules behaved like electric currents and that if electricity were mechanical in nature, then temperature should make no difference. The fact that it did indicated that the processes were not mechanical but probably chemical in nature.

Bernstein seized upon the dependence on temperature as a crucial argument for relating the electrical phenomena in nerve and muscle action to a chemical model of the underlying molecular events:[58]

58. Bernstein, *Elektrobiologie* (ref. 41), 90-91.

> If in muscles we are dealing with concentration circuits [electrical potentials resulting from concentration gradients], then according to the theory, not only must there be a positive temperature coefficient, but it must also turn out that the electromotive forces increase approximately in direct proportion to the absolute temperature, assuming that no change occurs in the constitution of the components with increasing temperature.

To test this hypothesis, Bernstein submerged a frog muscle in oil in a glass container. Recording electrodes and a thermometer were attached to the muscle. He put the container in a water bath and varied the temperature betwen 0° and 32°C, and found E to increase linearly with T (figure 25). In transforming Hermann's qualitative,

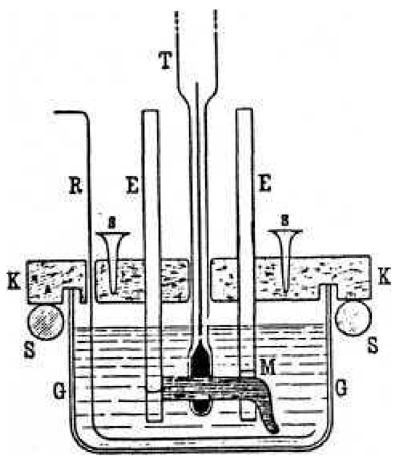

FIG. 25 Influence of temperature on the electromotive force in the muscle. The muscle M, immersed in oil in the glass container GG, is attached to clay recording electrodes EE, soaked in 0.6% saline solution and placed on the axial cross section (left side of the diagram) and on the surface (right side) of the muscle. A thermometer T records the temperature of the surface of the muscle. The temperature of the oil bath is varied by placing the glass container in a water bath. Bernstein, *Elektrobiologie* (1912) (ref.41).

phenomenological law into a quantitative one, Bernstein provided strong evidence for considering concentration gradients as the source of bioelectric potentials.

If concentration gradients were the source of bioelectric phenomena, Hermann's alteration theory and Bernstein's electrochemical theory could not be correct. Both explained the electrical phenomena as effects of biochemical mechanisms for energy transformation in nerves and muscles. Both supposed that part of the chemical energy generated by splitting and binding oxygen went into electrical energy in the electrolytes. But, as Bernstein pointed out, in current flow generated by concentration gradients, transformations involving chemical energy play no role. All that matters is the separation of already existing charged particles.

The mechanisms governing the membrane potential, the generation of action currents, and the transmission of the negative variation apparently did not depend upon the conversion of chemical energy supplied by the basal metabolic processes of nerves and muscles. This important consideration implied that the mechanisms for generating bioelectric currents differed from the mechanisms for producing contraction in muscles. The two systems were causally interconnected, since the depolarization of the membrane initiated contraction. But while intimately linked, they were not two components of the same system as required by the alteration theory or by Bernstein's earlier electrochemical theory.

Bernstein confirmed this consequence of his thermodynamic considerations with beautiful experiments on torpedo fish. The torpedo was relevant to deciding between the alteration and membrane theory because its electrical organs are for defensive purposes and have no connection to muscle movements. According to the alteration theory, the torpedo generates electrical potential by conversion of chemical energy much like a galvanic battery. The fish should therefore heat up when giving an electrical shock. The membrane theory, on the other hand, requires only the heat of reaction necessary to alter the membrane leading to its depolarization, and according to this theory this reaction heat must be negligible.

Bernstein tested this prediction experimentally by placing the isolated electric organ of the torpedo in an insulated container. A sensitive thermocouple was placed on the surface of the organ inside the container and connected to a mirror galvanometer calibrated to measure temperatures to 0.001 °C. The organ was stimulated from the nerve by an induction coil. The energy generated by the organ's electrical discharge was measured by nonpolarizing recording electrodes attached to it and connected to an electrical air thermometer. This instrument consisted of an electric bulb with a carbon filament. Current from the recording electrodes heated the filament, which heated the air, which heated a small capillary tube sealed into the bulb and containing a solution of alcohol and methylene blue, which expanded upon heating (figure 26). The capillary tube was empirically calibrated to measure temperature to 0.001 °C.

Here Bernstein made use of the general formula derived by Helmholtz from thermodynamic considerations to describe the voltage of a chemical battery. If U is the total energy of a system capable of a reversible isothermal process and T is the absolute temperature, then F, the "free" energy available for mechanical work, is given by:

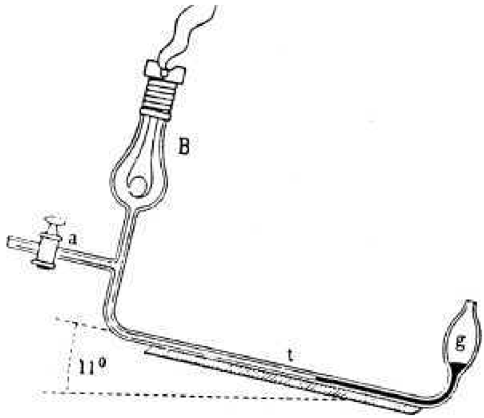

FIG. 26 Electrical air-thermometer. Current in the carbon filament of the bulb B heats air in the capillary tube extension. The capillary tube ends in a small bulb g, filled from a with a solution of alcohol and methylene blue. The temperature is read on the scale t. Bernstein, *Elektrobiologie* (1912) (ref.41).

$$F = U + T\frac{\partial F}{\partial T}.$$

This equation can be rewritten to describe the electrical energy available to drive a motor connected to a chemical source in a closed circuit with minimal resistance. With E in place of F and Q, the total heat liberated by the chemical energy of the system, in place of U, the expression becomes:

$$E = Q + T\frac{\partial E}{\partial T}.$$

In the case of the electrical fish, $Q = 0$. But in the experiment, Q is compounded of S_e, the heat produced by the external flow of current from the system as it does work in the electrical air thermometer; C, the heat given off by the surface of the organ inside the insulated box; and U, the heat liberated by the chemical process of alteration in the membrane leading to its depolarization:

$$Q = C + S_e - U.$$

Experimentation showed that despite the large voltages generated, the temperature of the organ itself increased by no more than 0.004°C. This contrasted sharply with the case of muscle action, where the rise in temperature is appreciable. Bernstein concluded that Q must be zero, and that the potential results from a concentration gradient. He ascribed the slight temperature increase to the chemical energy U needed to drive the alteration of the membrane. In several experiments, however, he detected a slight but noticeable cooling. In thermodynamic terms, the organ was operating like an endothermic battery, absorbing heat from the surroundings and converting it to electrical energy. Bernstein reasoned that when *U* does not suffice to discharge the organ, heat is absorbed from the organ itself in order to drive the mechanism.

Nernst's model required the presence of a semipermeable membrane. Ostwald had suggested in 1890 that an "ion sieve" would probably turn out to be applicable to bioelectric currents, but his idea had not been pursued.[59] By then it was widely known that semipermeable membranes produce certain electrical phenomena in plants. In 1873 Pfeffer had provided experimental evidence indicating that the plasma membrane in plant cells is semipermeable.[60] Bernstein now gathered these various threads together and supposed that the membranes of blood, nerve, and muscle cells in animals are semipermeable.

Support for this view was provided independently by Overton, who showed that the cell membranes of animal tissues are composed of lipids.[61] He demonstrated that a monolayer of lipids artificially prepared in the laboratory is semipermeable to certain dyes while impermeable to other dyes and a variety of other molecules. He showed furthermore that the dyes capable of penetrating the lipid layer are also capable of penetrating muscle and nerve membranes. He inferred that muscle and nerve membranes are semipermeable. By 1912, it had become clear on the basis of work done by Rudolph Hober that plasma membranes are composed of both proteins and lipids.[62]

Integrating it all, Bernstein noted:[63]

> Ostwald's earlier suggestion could now be given a tangible form, for the plasma membranes on the outer shell of the muscle (sarcolemma) and nerve fibers (neurolemma) could be viewed as such a semipermeable membrane, leaving undecided the question whether a similar structure pervades each individual fiber....If on the interior of such a membrane an electrolyte is contained in greater concentration C1, than its concentration C_2 on the outside of the membrane,...a potential is generated across the surface of the membrane. . . . If one imagines . . . that the

59. W Ostwald, "Elektrische Eigenschaften halbdurchlassiger Scheidewande," *Zeitschrift fur physikalische Chemie, 6* (1890), 71-82.

60. Pfeffer, in *Pringsheims Jahrbucher fur wissenschaftliche Botanik 9* (1873) 308-326. Discussed in Biedermann (cf. ref. 39), *2* (1895) 451- .

61. *E Overton, "Beitrage zur allgemeinen* Muskel- und Nervenphysiologie," *Pfluger's*

62. Bernstein (ref. 41), 102.

63. Ibid., 93.

> positive ion will be admitted by the membrane while the negative ion is held back, then the membrane will be polarized; for the positive ions attempt to diffuse outward but are held in check by the negative ions in the interior.

Bernstein showed that as long as the muscle remains intact no current will flow. If the permeability of the membrane were suddenly to change, either through injury or some physiological mechanism, allowing the ions contained within the plasma to escape, the cell will depolarize, giving rise to DuBois-Reymond's "muscle current."

The new membrane theory offered a plausible explanation of the central phenomena of nerve and muscle action, including the effects of electrotonus (figure 27). But even more important, it provided for the first time **a** means for deciding *quantitatively* which electrolytes were

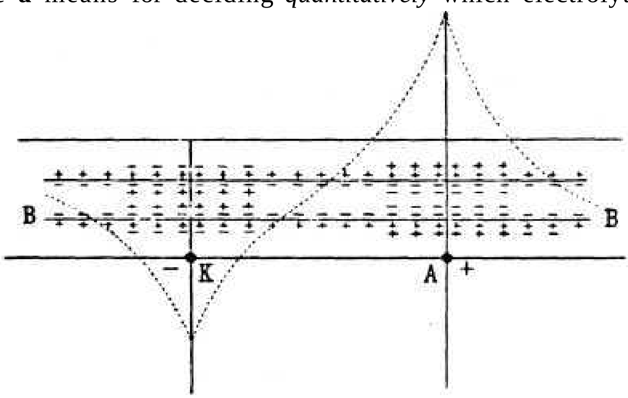

FIG. **27** **Electrotonus according to the membrane theory.** The phenomena connected with electrotonus result from the movement and accumulation of differently charged ions on both sides of the membrane BB. The positive ions in the external electrolyte migrate to the anode where they add to the positive ions normally present on the exterior of the membrane. The negative ions in the internal electrolytes migrate to the anode. The impermeability of the membrane thus leads to an increase in the potential near the anode. Negative ions in the external medium migrate to the cathode where they bind with the positive ions normally present on the membrane exterior. On the interior of the membrane near the cathode, positive ions in the interior electrolytes bind to the negative ions normally present on the interior of the membrane, thus lowering the potential in the region near the cathode. Bernstein, *Elektrobiologie* (1912)(ref.41).

essential in generating action potentials. Substituting the experimentally determined concentrations of the electrolytes known to be present in muscle plasma and in the surrounding fluids, Bernstein chose potassium as a likely candidate. The available experimental determinations gave a 5.2% concentration of K_2O on the muscle interior and a 0.26% concentration in the extra-cellular fluid. This led to a value of not less than 68 millivolts for the potential difference, a value close to that required for the action current. An alternate candidate appeared to be sodium ion. In its case experiment indicated a concentration of 0.42% in the external fluids and a concentration of 0.09% in the muscle interior, leading to a value of between 31 and 45 millivolts for the membrane potential.

These numbers suggested two possibilities to Bernstein. Either the potential is generated by the semipermeability of the membrane to potassium alone, or both electrolytes played a role, with sodium concentrated on the outside of the membrane and potassium on its interior. In the final version he opted for a single electrolyte, potassium.[64] To buttress the hypothesis of the action of potassium, Rudolph Hober showed that by increasing the concentration of potassium in the surrounding fluids, he could reduce and even reverse the resting potential of the muscle.[65] This indicated that in normal conditions the membrane retained excess potassium on the interior.

There remained the alteration theory, which likened the transmission of the action wave to a rapid "inogenic" cycle of chemical transformations of the muscle tissue along the cross section similar to that observed in the onset of rigor mortis. Electrochemically, the mechanism had to be associated with the production of a negative electrolyte along the cross section. Accordingly, the alteration theory leads to the expectation that the greatest potential difference ought to be measured between the surface of the membrane and the cross section of the nerve or muscle. The membrane theory, however, does not assume the production of a negative electrolyte through some biochemical process of dissociation within the muscle at the point of stimulus; rather, like Du-Bois-Reymond's original theory, the new membrane theory assumes a potential difference between the inner and outer surface of the membrane *before* the stimulus. Accordingly, the membrane theory predicts that the greatest potential difference will occur along the external surface, that is, along the membrane. This gave rise to a crucial experiment.

The basic features of the experiment derived from observations made by Hermann himself. He found that when two halves of an intact muscle are subjected to different temperatures, current flows from the warmer to the colder part. This observation can be predicted by the membrane theory. In this case, the concentrations K_1 and K_2 in the Nernst equation can be regarded as identical, so that the potential is E = const. T_1 for the part of the muscle having temperature T_1, and similarly for the half with temperature T_2. The current generated by the temperature difference is proportional to $E_1 - E_2$ and hence to $T_1 - T_2$. Bernstein's measurements confirmed that currents are directly proportional to the temperature differences

64: Ibid., 98-99.

(figure 28).

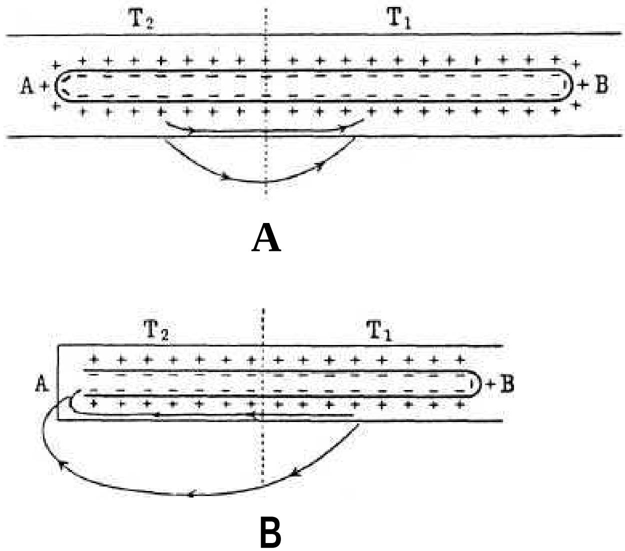

FIG. 28 Experimental comparison of **the** membrane **and** alteration theories. In A, the left half of an uninjured muscle AB, with its membranes intact, is placed in a heated oil bath. The left half of the muscle assumes a temperature T2 > T1 of the right half. Recording electrodes indicate an increase in the potential directly proportional to the difference in temperature as predicted by the membrane theory from the Nernst equation. In B, the same experiment is repeated with an injured muscle. Recording devices attached to the axial cross section on the left and the surface on the right half measure the resting current. In this case heating the left half has no effect on the measured resting current of the muscle, contrary to the alteration theory. Heating or cooling the right side does increase or decrease the measured current. As predicted by the membrane theory, the change is proportional to the difference, T_1- T_2, of the temperature of the muscle halves. Bernstein, *Elektrobiologie* (1912) (ref.41).

When the current between the surface membrane and the cross section is measured in an injured muscle, heating the membrane surface increases the electromotive force whereas cooling the membrane decreases it, in direct proportion to the absolute temperature. Heating and cooling the cross section, however, has no effect on the electromotive force. From these results Bernstein concluded that "the greatest potential difference does not appear along the cross section as assumed by the alteration theory, but rather along the surface as required by the membrane theory."[66]

6. INSTRUMENTS AND CONCEPT FORMATION

The development of theories of bioelectric currents illustrates several important features about the process of theory construction in experimental disciplines. It indicates the role of experiment and the refinement of technique and instrumentation. Crucial to the historical development were instruments for recording bioelectric currents. The principal phenomena of the domain, such as the "muscle current," "nerve current," negative variation, and electrotonus were defined by the instruments that made them accessible. To press deeper, it was necessary to refine this first generation of instruments. Bernstein's rheotome and other instruments, such as DuBois-Reymond's nonpolarizing electrodes, Helmholtz's electro-tuning fork, Bernstein's acoustical interrupter, the electrical air thermometer, and others not discussed here, refined the relationships roughly revealed by the first generation of instruments.

These refined instruments added support for DuBois-Reymond's hypothesis that the curve represented by his multiplicator as the negative variation was in reality a summation of discrete events. Furthermore, they led to a more detailed understanding of the negative variation. Whereas DuBois-Reymond suggested that a more sensitive instrument might reveal discrete "spikes" extending below the abscissa line representing the "muscle current," Bernstein's rheotome established the maximum magnitude of the negative variation and its relation to the resting current. With the rheotome Bernstein was also able to establish that the negative variation precedes the onset of muscle contraction and that the negative variation has run its course before the muscle contraction begins. Later, when he developed the membrane theory, this finding would support the implications, derived from thermodynamic considerations, that the mechanisms involved in generating and transmitting bioelectric currents must be distinct from the mechanisms associated with muscle contraction.

The advances made through the refinement of instrumentation did not depend upon any technological breakthroughs. The instruments that led to the developments described here, from 1843-1902, were modifications and variations of a generic technology supplied by the electrotechnical industry of the early 1840s and 1850s. This suggests that the concepts involved in theory construction were related to the available technology, that concept elaboration and theory construction may form part of a web including the available material culture. If that is the case, the interpretation of the phenomena connected with bioelectric currents depended on the conceptual frameworks from which the technology employed in investigating them derived. Thus, DuBois-Reymond's formative hypotheses were all guided by the search for analogies between bioelectric currents and the conceptual models developed by Faraday, Ampere, Ohm and others. DuBois-Reymond's initial concepts derived directly from the instrumentation used by Faraday in investigating induced current.

Instrumental limitations on concept formation

The available instruments also limited the range and content of concepts and theories. Consider the concept of the membrane potential itself. By the early decades of this century sensitive electrometers, such as the capillary electrometer and ultimately the oscilloscope, made it possible to record the firing of individual nerve and muscle cells. But before the development of intracellular recording and stimulating electrodes between 1939 and 1946,[67] it was not possible to make a direct measurement of the membrane potential of individual nerve or muscle cells. Before these technical developments, determinations of potential were made by activating the nerve or muscle either by stimulating it or altering the membranes in some way, e.g., by chemical or heat shock, and by using a compensating current of known voltage shunted through a sliding-wire resistor to measure the voltage of the muscle.[68] With intracellular recording and stimulation a more detailed picture of the processes connected with changes in the membrane potential could be obtained. The potential could be gradually adjusted from within the cell, bringing the membrane to the threshold or "action potential," allowing changes transpiring when the membrane depolarizes to be followed in detail. This picture was further enhanced through the introduction of radioactive tracers for following the ion flows.

Certainly one result of such refined instrumentation and technique would have been to force a revision of Bernstein's single electrolyte model. Researchers would have found that upon depolarization the membrane potential is not only abolished but reversed. Evidence supporting a brief reversal of polarity upon triggering the action current had been presented by Burdon-Sanderson and Gotch in a paper published in 1891, but Bernstein dismissed it as an artifact, holding that the true value of the membrane potential could be determined only

67. The relevant stages in the development of intracellular recording and stimulating electrodes can be followed in A.L. Hodgkin and F.A. Huxley, "Action potentials recorded from inside a nerve fiber," *Nature, 144* (1939), 710-711; H.J. Curtis and K.S. Cole, "Membrane resting and action potentials from the squid giant axon," *Journal of cellular and comparative physiology, 19* (1946), 135-144; and J. Graham and R.W. Gerard, "Membrane potentials and excitation of impaled single muscle fibers," ibid., *28* (1946), 99-117.

68. See Bernstein (ref. 41), 7-8.

immediately upon damaging the membrane.[69] Bernstein's experiments with the rheotome had led him to conclude that the action current or negative variation is always equal to and never greater than the membrane potential. The single electrolyte model fit beautifully with this finding.

If it were admitted, however, that upon depolarizing the membrane the interior of the nerve or muscle cell suddenly and briefly becomes positive, as is in fact the case, then a second electrolyte, sodium, would have to be included in the cast of characters. This could not be done merely by introducing a second electrolyte and arguing that depolarization of the membrane renders it briefly permeable to sodium ions. That might explain the reversal in polarity, but it created the problems of providing a mechanism for transporting sodium back out of the cell against its concentration gradient, and of providing a mechanism for preventing the resting potential of the membrane from running down as a result of the diffusion of potassium and sodium ions across the membrane. The solution to these problems required an energy-consuming, *active transport* mechanism, the so-called sodium pump.

But the power of the ion model lay precisely in its ability to interpret nerve and muscle membranes as ionic batteries that do not depend upon the active conversion of chemical into electrical energy. The introduction of a second electrolyte would imply a more complicated picture requiring a deeper understanding of the biochemistry of metabolic pathways than the ionic models derived from Nernst could deliver. While perfectly suited to exploit the most powerful implications of current work in physical chemistry, Bernstein's instruments placed limitations upon and implied definite choices among the concepts that could be elaborated in constructing a theory of bioelectric phenomena.

That conceptual development in science, particularly within experimental disciplines, should be closely tied to the state of the available technology and technique seems obvious. Scientists do not lack imaginative hypotheses. The transformation of such hypotheses into workable empirical concepts is dependent on the available technical means, which may not be adapted equally to all the hypotheses.

A case in point is Hermann's alteration theory, which referred the transmission of nervous impulses and action waves to metabolic pathways in nerves and muscles. This hypothesis could not be applied effectively during the 1860s and 1870s when Hermann proposed and defended it. By the mid-1930s, however, with the enormous advances

69. Ibid., 105-106.

then being made in biochemistry, the time was ripe for an approach similar to Hermann's. Investigating how the ions that escape through a membrane during excitation are recovered, several people showed that during anoxia the excitability of crab nerve can be maintained in the presence of phosphopyruvic acid, adenosintriphosphate, and thiamin, the components of the glycolytic cycle. Rudolph Hober speculated that this cycle might be an energy source needed to drive the process of restoring and maintaining the resting potential[70]

A further addition to the theory of action potentials during the late 1930s and 1940s also falls within the general conceptual framework Hermann and Pfluger tried to develop but could not for lack of appropriate technical means. By the mid-1940s it appeared that factors other than inorganic ions might alter the constitution of the nerve and muscle membranes and thus permit ionic flow through the porous membrane channels and a depolarization of the cell, as proposed in Bernstein's electrochemical model. Hober suggested that nerve membranes may contain "split products possibly capable of acting as 'precursors' to elicit reversibility, and in small amounts, strong bioelectric currents."[71] In much the same vein as Hermann had once speculated, Hober proposed that a chemical wave precedes the depolarization-repolarization of the nerve and that essential components of this chemical wave might consist of the liberation and reinsertion of acetylcoline and thiamin as the precursors.[72]

My concern is not to argue that modern theories are revivals of Hermann's ideas. His concepts were imprecise, and he had no way to identify the elements of his suppositious "inogenic" pathway in nerves and muscles. With the powerful methods of the biochemistry of the 1930s and 1940s, however, the concept of an energy-generating pathway was developed into an empirical concept capable of solving problems in the old membrane theory: the repolarizaion of the membrane and reversal of the potential during the passage of the action wave, phenomena which did not fit into the classical picture of the nerve derived from Bernstein's work. The shape of the concept and the role it played in the progressive development of theory depended upon the state of the technical means for elaborating and rendering it empirically valuable.

70. Hober, "The membrane theory," New York Academy of Sciences, *Annals, 47* (1946), 386-387.

71. Hober, *Physical chemistry of cells and tissues* (Philadelphia: Blakiston, 1945), 351.

72. Hober, ibid., 352, and 387-389.

Models and theory construction

The interdependence of experimentation, instrumentation, and conceptual models points to a second feature illustrated by this historical case: that new research fields are highly dependent for their theoretical constructs on a pool of models made available from other successful research areas. Between DuBois-Reymond's initial theorizing and the emergence of Bernstein's membrane theory, neurobiology advanced by adapting to its own special requirements models drawn from electricity, structural and organic chemistry, and energetics and physical chemistry.

Models play a crucial role in utilizing the available resources of both the material and intellectual culture in order to articulate concepts. DuBois-Reymond's attempts at modelling were very important; they brought the first semblance of order into the domain and his approach to developing models for new data domains is a textbook example of good practice.[73] Starting from a point where the only theory available was speculation on the analogy between electricity and the "nervous fluid," DuBois-Reymond focused on a single relationship indicated by his instruments: the conditions for the detection of the muscle and nerve currents. Selecting this one very specific relationship, he built a model, in this case a physical one, which localized the structure of his data and around which other supplementary data could be assimilated. Since the relationship DuBois-Reymond chose was directly analogous to Faraday's laws for induced current, structural elements of the models for nerves and muscles could be elucidated by concepts derived from electricity and magnetism. Further experimentation revealed only a partial correspondence between these two domains, however. This led to a second round of modelling, particularly to assimilate new data concerning electrotonus. These new models were based on concepts being explored in the fledgling new area of physiological chemistry, and the attempt to incorporate their best features along with successful parts of DuBois-Reymond's models led to progress in the construction of testable concepts specific to neurobiology.

A further point of significance concerns the difference between types of models. DuBois-Reymond's models were not only conceptual models, dependent on the syntax and conceptual elements of a better known system; they were also *physical* models. DuBois-Reymond's

73. Cf. J.W.L. Beament, ed., *Models and analogues in biology* (New York: Academic Press, 1960), and Leo Apostel, "Towards the formal study of models in the non-formal sciences," in Hans Freudenthal, ed., *The concept and role of the model in mathematics and natural and social sciences* (Dordrecht and Boston: Reidel, 1961), 1-37.

instrumentation prevented him from exploring the properties of nerves and muscles at the microscopic level. But the drive to construct an explanatory model for the phenomena carried beyond the macro level. No matter how finely he sectioned muscle and nerve segments, the nerve and muscle currents always behaved in the same way. To go beyond segments to individual muscle fibers and even to fiber elements, DuBois-Reymond showed, by experimenting on his models composed of unit voltaic cells, a similarity between his model and the physiological system he was investigating. This standard practice in biophysics has been employed with success in analyzing the functional properties of membranes and muscles by constructing artificial membranes and actomyosin threads that behave like living muscles and membranes.

The case we have explored illustrates the heuristic role of more abstract conceptual models, particularly analog models and formal analogies. Pfluger's model of nerve mechanics reveals the heuristic power of formal analogies. Not intended as a physical analog of nerves or muscles, it clarified relationships between key phenomena in terms of the conceptual structure of energetics as the primary logical apparatus. The model prepared a synthesis of DuBois-Reymond's para-electric model with physiological models deriving from physiological chemistry. The treatment of potential gradients by Nernst and Ostwald functioned as a conceptual analog model for refining Pfluger's formal analogy. Interpreting Pfluger's model in terms of the thermodynamic analysis of diffusion potentials and ion concentration gradients smoothed the way for a theoretical model of bioelectric currents, the membrane theory, constructed by analogy to ion batteries. Once its value for ordering the principal phenomena of the domain had been demonstrated through experiment, a number of workers made physical models of membranes.

A crucial step in the refinement of theory was physical interpretation of the conceptual model. To test Overton's concept of the membrane as a monomolecular layer of lipids, ionic movements across solvent membranes constructed from olive oil, oleic acid, and diamylamine were compared with their movements in physiological membranes. By experimenting with gels of various porosity, Hober amended Overton's model by adding proteins to lipids to represent cellular membranes as "lipoid-sieves." As this example indicates, there is a dynamic, dialogic relation between different types of conceptual and physical models, as well as between experimentation, instrumentation, and the construction and refinement of models. These aspects of model-building reveal the construction of adequate empirical concepts and theory as a process of bootstrapping via partial correspondences and homologies to domains whose concepts are more stable.

Quantitative models and causal stories

Research fields enter into a mature phase when they make the transition from the construction of qualitative to quantitative models. The importance of a quantitative model does not lie in attaching numbers to the outcomes of measurements and experiments. As we have seen, DuBois-Reymond, Helmholtz, Bernstein and others all made measurements (some of them, such as Helmholtz's determination of the speed of stimulus transmission, being very exacting) long before the field was guided by a quantitative model. Rather, the transition to a quantitative model is an essential step in constructing a detailed *causal story* about the interrelationships between the phenomena of the domain.[74] The strength of the membrane theory was that it could draw upon thermodynamic models of the underlying causal processes to establish precise quantitative relationships between parameters subject to experimental determination. This made possible for the first time a means of identifying the relevant phenomena and their interrelationships as well as the fundamental entities of the research domain. The Nernst equation and the Helmholtz equation for the conversion of chemical to electrical energy offered an interpretation of the phenomenological laws concerning heat in nerve transmission and muscle contraction. Experimentation guided by the Nernst equation permitted determination of the electrolytes that generate the membrane potential.

The Helmholtz equation clarified the causal relationships between the generation of the membrane potential and the metabolic processes connected with muscle contraction. The application of this particular quantitative relation led to the surprising result that bioelectric phenomena were causally related to, but separate in origin from the mechanisms for generating muscle contraction. This refinement of the causal story helped in turn to clarify phenomena connected with the negative variation, for example, why the negative variation runs its course before the onset of contraction. The quantitative features of Bernstein's membrane theory permitted the unequivocal demonstration that nerve and muscle action are causally dependent on processes of an electrical character. This demonstration had eluded all previous attacks on the problem.

74. Cf. Nancy Cartwright, *How the laws of physics lie* (Oxford Univ. Press, 1983) 4-15, 143-162, esp. 161-162.