SECTION II

Electrophysiology in the 19th Century

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The impact of Volta's discovery in science was felt very soon. In 1818 Hans Christian Oersted [1777-1851] a Danish physicist, discovered electro-magnetism by showing that a magnetic needle, a compass, was deflected if placed near a wire conducting an electric current. Only two years after Oersted's discovery, the first practical device to measure the flow of electricity through a conductor was invented by Schweigger in Germany. The wire carrying the electric current was coiled a number of times around the magnetic needle, thus reinforcing or multiplying the effect of the current. This instrument was initially called a rheometer or a multiplier and later, a galvanometer, name under which it became commonly known. Five years after the invention of this device, another Italian, C. Leopold Nobili [1784-1835] developed a more sensitive galvanometer and revindicated Galvani demonstrating with the aid of the new instrument that muscles, upon twitching, do generate electric currents. Neither Galvani nor Volta had been totally right or completely wrong. There existed, after all, an animal electricity.

An staunch defender of Galvani was Alexander von Humbold [1769-1859], the prominent German geologist, naturalist and explorer who repeated both Volta's and Galvani's experiments and designed others himself. He reached the conclusion that Galvani had discovered both the bimetallic current and the existence of animal electricity.

From the beginning of the 19th Century the newly invented multiplier or galvanometer was applied to the study of a variety of physical and physiological problems. In particular, among the latter were the electrical activity of nerve and muscle. After Nobili, using his astatic galvanometer, announced that muscle generates electric currents upon contraction other investigators rushed onto the stage. Prominent among them was another Italian.

A. Electrophysiology in Italy

Carlo Matteucci [1811-1868]

Professor of Physics in Pisa as well as one of the first Italian ministers of Public Instruction, Matteucci discovered that resting, uninjured muscles behave as a source of electric current. His instruments were sensitive enough to detect a decrease in the amplitude of such a current during a "tetanic" contraction (term that he introduced to denote the repetitive high frequency twitching of muscle induced by tetanus toxin.

Matteucci was a prolific observer. He showed that the nerve of a nerve-muscle preparation (a muscle with its motor nerve attached to it) could be stimulated by another muscle if in close contact with the nerve. He was, in fact, the first physiologist who used the contraction of a muscle attached to its motor nerve as a way to detect stimulation, or excitation, of the nerve. The muscle serves as a convenient indicator of nerve activity. Although Galvani's first experiment consisted just of this, he did not fully realize the significance of dealing with two different excitable tissues in series, using the second or the muscle to find out what was happening in the first, the nerve. Matteucci also noted that excitation originated at the cathode (or negative electrode) when the stimulating circuit was closed, and at the anode (or positive electrode) when the circuit was opened. In addition, he noticed that the stimulating electrodes became polarized (i.e. develop a potential of their own) following the flow of current through them.

Matteucci was first to observe the phenomenon of electrode polarization. In the words of a 19th century physiologist, this could be described as follows: if a galvanic current or dc is made to flow between two metal electrodes joined by a moist conductor and the two wires are rapidly disconnected from the source of current and connected to the galvanometer, the anode, i.e. the electrode that was connected to the positive pole with respect to the cathode (or electrode connected to the negative pole) will continue to be positive for a certain time. It was noticed, furthermore, that the same phenomenon was apparent if the current was applied through non-polarizable electrodes. This showed that in addition to the electrodes, the preparation itself (nerve or muscle) had become polarized. Somehow, the flow of current had altered the state of the preparation, so that, even when the externally applied current had ceased to flow, a potential difference was momentarily established between the two points of the tissue where the electrodes had been applied.

B. Electrophysiology in Germany

The School of Johannes Müller

In the 1840's the center of electrophysiological research shifted from Italy to Germany, where a flourishing school of Physiology had been founded by Peter Johannes Müller [1801-1858] (Fig. 1), Professor in Bonn first and later in Berlin. His students included scientists of the caliber of Emil Du Bois-Reymond [1818-1896] (Fig. 2), originally from Switzerland; Hermann von Helmholtz [1821-1894] who began his career as a military physician and ended it as Professor of Physics in Berlin; von Brücke, and Sechenov, later regarded as the father of Russian Physiology.

Figure 1. Johannes Müller

Emil Du Bois-Reymond [1818-1896]

Although interested in electricity Müller regarded its physiological effects as little more than interesting artifacts, but he had the foresight to direct one of his best students to the new field. Thus, in 1842 Emil Du Bois-Reymond received from his professor a copy of one of the first works of Matteucci, his *Essai sur les phenomenes electriques dans animaux* (Essay on electrical phenomena in animals) (Paris, 1840), together with the suggestion that it would be interesting to confirm and expand the Italian's work. Du Bois-Reymond followed Müller's advice so literally that by November of the same year he had already published a preliminary note on the subject and by the end of the decade his monumental work entitled *Untersuchungen über thierische Elektricitat* (Investigations on animal electricity) was published in two volumes, in 1848 and the second in 1849 (1) (Fig. 3).

Figure 2. Emil du Bois-Reymond

Du Bois-Reymond was an excellent experimentalist with great skills in instrumentation. It did not take him long to repeat Mateucci's observation on the current that flows in an injured muscle. He called this *Muskelnstrom*. Moreover, he detected, using faradic stimulation (i.e. a current produced by an induction coil, that the *Muskelnstrom* decreased when the motor nerve was stimulated tetanically and named this a "negative variation" or "action current" of muscle. He used the term negative in an algebraic rather than an electrical sense, to

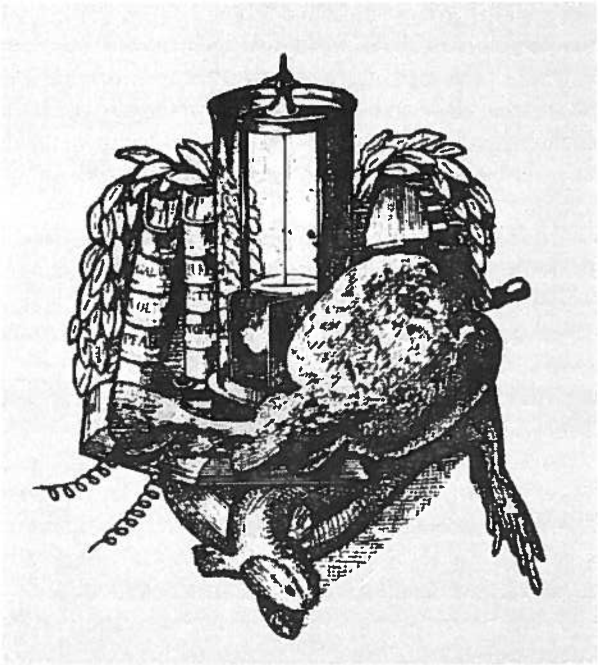

Figure 3. Frontispiece: "Untersuchungen Über Thierische Elektricität". Shows Nobili -du Bois-Reymond Galvanometer; static electric generator; an electric fish, *Torpedo*; electric eel; frog leg; and books by Galvani, Volta, *et al.*

mean that the amplitude of the recorded current decreased.

But he went even further. By connecting his sensitive galvanometer, with a total length of wire of more than 5 Km., coiled some 24,000 times around the core, he discovered, in 1845, the existence of similar electrical phenomena in nervous tissue. He described how persistent electromotive changes were recorded from excised nerves and observed that if the leads of the galvanometer were applied to the surface of the nerve, far from the cut ends, this surface was equipotential. However, if one of the leads was placed close to the cut end and the other further away, the lead nearest to the cut end was negative with reference to the other.

He also noticed that any injury produced by heat or compression etc., on a previously intact region of the nerve would make it negative with respect to the nearby regions. Since these currents appear in nerves that may be regarded as unexcited, they were called currents of rest. In contemporary terminology they would be described as injury currents produced by the resting potential of the nerve fibers discharging through a damaged region of their excitable membrane. In addition, Du Bois-Reymond was able to show that, upon stimulation, the nerve undergoes a negative variation similar to that observed in muscle. In so doing, he discovered the nerve impulse or action potential. He claimed, and rightly so, that he had succeeded in demonstrating the identity of the 'nervous principle' with electricity. Two close associates of Du Bois-Reymond, von Helmholtz and J. Bernstein, went still further: the former was able to measure the speed or rate of propagation of excitation in frog nerves, and Bernstein confirmed von Helmholtz' results and, using a different technique, he determined the shape or time course of the nerve action current.

One of the most important result of his studies was the discovery by Du Bois-Reymond that such 'electromotive' changes, i.e. the polarization of the excitable tissues, occurred not only at the points where the current was applied and the region between them, but, appeared also, in the 'extrapolar' regions, at considerable distances from the electrodes, during as well as following, the application of a current. That is, if two points of the extrapolar anodic region are connected to a galvanometer, the point closer to the anode (but some distance away from it) would be more positive than the point further away. This phenomenon is the so-called 'anelectrotonus' and the opposite one, which occurs at the cathodal region is known as 'catelectrotonus'. Both phenomena were named by Du Bois-Reymond 'electrotonus'. This term and his ideas concerning the mechanism of the origin of the polarization were based on the analogy of the lining up of elementary magnets in Weber's theory of magnetic induction. Thus, Du Bois-Reymond conceived that the axoplasmic core of a peripheral nerve fiber was longitudinally polarizable.

Hermann von Helmholtz [1821-1894]

He was born in Potsdam (Fig. 4), the eldest son of a grammar school master. He attended school in his hometown and, as a cadet, he studied medicine at the "Royal Friedrich Wilhelm Institute for Medicine and Surgery" in Berlin, where he became a pupil of Johannes Müller. From 1843 until 1848 he worked as a military doctor in Potsdam where he passed his state medical examination. While in Potsdam, he joined the newly founded Berlin Physical Society and conducted joint research with Johannes Müller on the origin of the nerve fibers from ganglion cells. In addition, he carried out studies on the so-called 'vital force', a subject that at that time attracted the attention of physicists, chemists and biologists. The result of these studies was a classical paper on physics, which appeared in 1847, entitled *Über die Erhaltung der Kraft* (On the conservation of energy) in which he applied in a mathematical form the energy principle established by Robert Mayer [1814-1878] and James Joule [1818-1889] to all areas of Physics, including Michael Faraday's law of electrical induction.

Figure 4. Hermann von Helmholtz (1821-1894)

In 1848, Helmholtz began to lecture at the Berlin Academy of Arts and one year later he was appointed Professor of Physiology and Pathological Anatomy at the University of Königsberg. There he carried out his famous experiment on the velocity of the nerve impulse and invented the first instrument which allowed the study of the retina in the living eye (2). In 1858 he went to Bonn as Professor of Anatomy and Physiology; in 1868 to Heidelberg as Professor of Physiology. In 1871 he was appointed to the new chair of Physics in Berlin, although he had never studied Physics formally. Finally in 1888, at the age of 67, he took over the presidency of the newly founded "Physikalish-technische Reichsnstalt" in Berlin, where he remained until his death. Helmholtz's contributions to science were widely recognized. He became a member of the most important scientific academies and societies and in 1882 Kaiser Wilhelm I raised him to the nobility (his name becoming von Helmholtz). His contributions had an impact on almost every branch of science.

During his stay in Königsberg he was oriented towards sensory physiology, the psychology of which had interested him as much as the purely biophysical aspects. With the help of his ophthalmoscope and ophthalmometer he was able to measure the curvature of the cornea and the lens, as well as the exact distance between those two surfaces. He also clarified the problem of the lens accommodation and succeeded in determining the function of the retinal rods and cones, discovering that the macula lutea is the site of highest light sensitivity and acutest vision.

In 1853, Helmholtz formulated the differential equations for the distribution of potential in bodily conductors, thus setting the foundations for the development of vector electrocardiography some 100 years later. In Bonn he worked on physiological optics and acoustics, areas in which he published two outstanding monographs: *Die Lehre von der Tonempfindung als Grundlage für die Theorie der Musik* (Doctrine on Tone Reception in Music Theory) (1862), and the *Handbuch der physiologischen Optik* (Handbook of Physiological Optics), the latter in several volumes from 1856 to 1866, and generally regarded as a standard work in sensory physiology.

From our viewpoint, his short communication on the conduction velocity in nerve can be regarded as the starting point of modern neurophysiology and biophysics. The early physiologists, including Johannes Müller, believed that the function of nerves was to serve as pathways for the propagation of an immeasurable agent: the so-called *Spiritus animalis*. Even as late as 1844, Müller had predicted explicitly that one would never be able to determine the "velocity of nerve action" as he regarded the time-scale involved to be infinitely small. However, Helmholtz established in his 1850 communication that a finite time is necessary for the excitation caused by a short electric current to travel from the nerve plexus to the end of the nerve into the muscle. On January 15th (1850), Helmholtz sent this short report to his friend Du Bois-Reymond in Berlin, with the request that he submit it to the Physical Society "to deposit it in their files to safeguard its priority". At the same time copies of it were sent to Johannes Müller and Alexander von Humboldt for the Berlin and Paris Academies respectively. On January 21st Johannes Müller presented Helmholtz's report as the first communication in the sitting of the physicochemical group of the "Royal Prussian Academy of Sciences". From von Helmholtz's data it appears that the conduction velocity of frog nerve was of between 25 and 43 m/s; a result amazingly accurate when compared with measurements performed with present techniques.

In a subsequent investigation, Helmholtz refers to the importance of temperature. He mentioned that he had obtained the lowest values for conduction velocity in nerve on cold days, a fact that speaks for his exact and critical manner of work. He described his experiments in a concise communication of less than two pages (1850). To measure the velocity of propagation of the action potential, he used an ingenious technique by means of which he could determine the instant when a circuit was closed or opened. Closing of the circuit was produced by the stimulus applied to the nerve. And the circuit was opened by the ensuing muscle contraction. By placing the stimulating electrodes at different points on the nerve, Helmholtz was able to calculate that the wave of excitation was traveling at a velocity of 27-30 m/s. With this technique it was also possible to determine that when the two leads of the galvanometer (and we shall call them "electrodes" from now on) were placed over an intact region of a nerve or muscle, a diphasic deflection would be recorded upon stimulation. On the contrary, if the electrode more distant from the point of stimulation was placed on the cut end of the preparation, one would observe a monophasic deflection superimposed on the negativity due to the nerve current. This observation foreshadowed the so-called "overshoot" of the action potential, that was not re-discovered until the 1930's (cf. Grundfest (3). It is interesting to note that the techniques that Helmholtz used were actually a spin-off from the technology developed by Prussian militarism. An important problem for the mid-19th century artillery was to calculate the flight path of a shell. For this it is important to know its velocity and the time needed for the powder mass to ignite.

In a lecture he gave in Königsberg on December 13th, 1850, entitled *Über die Methoden Kleinste Zeittheile zu messen und ihre Anwendung zur physiologischen Zwecke* (On the methods to measure very small time intervals and their physiological applications), von Helmholtz traced the history of the measurement of small time intervals, describing the instruments employed at that time, in particular the Siemens rotating cylinder which allowed the measurement of times as short as 40 ms and the apparatus 77ms could be measured. He remarked "...as you see the microscopy of time has greatly surpassed that of space". Not surprisingly the speed of sound was measured at about this time. Helmholtz's work on the velocity of conduction in nerve did not finish with his 1850 report. His full paper on the subject was published in 1852 (4). The average given in this paper was of 27.25 ms.

Julius Bernstein [1839-1912]

He was born in Berlin on December 8, 1839 (Fig. 5), the son of the Jewish theologist, author and politician Aaron Bernstein. The elder Bernstein was keenly interested in the natural sciences and had turned his apartment into a chemical and photographical laboratory. He devoted himself to the production of stereophotographs and had solved the problem of two way speech in one wire. He had the ability to present complicated subjects in a form understandable to all and had made a name as a scientific publisher. His son Julius helped him with the experiments.

Bernstein began his medical studies in Breslau in 1858 and soon became interested in physiology under the influence of Rudolf Heidenhain [1834-1899]. Through his childhood friend Ludimar Hermann [1838-1914] he gained admission into Emil Du Bois-Reymond's laboratory in Berlin, where he worked for several years. In 1864, he became an assistant to Helmholtz in Heidelberg and he obtained his M.D. degree with a dissertation on invertebrate muscle physiology in 1867.

Du Bois-Reymond's influence on Bernstein in the area of bioelectricity was continued by Helmholtz. After Helmholtz was appointed to the chair of physics in Berlin, Bernstein took over the direction of the Heidelberg Institute as his substitute, including his lectures. However, he soon returned to Berlin where he stayed until 1872. In that year he was appointed to the chair of physiology in Halle, as a successor to Friedrich Leopold Goltz [1834-1902] who had moved to Strassburg. Bernstein worked

Figure 5. Julius Bernstain (1839-1917)

in Halle for 46 years until his death on February 6, 1917. Bernstein's interests extended beyond physiology into the areas of Physics (particularly Electricity) Molecular Physics, Thermodynamics, Physical Chemistry, Mathematics and Astronomy. His lectures and experimental demonstrations were carefully prepared. In addition to the six main lectures per week, and the four-hour laboratory session, he often held special lectures where recent papers were discussed. He wrote an excellent textbook of Physiology which influenced more than one generation of muscle physiologists.

Bernstein's research work extended over vast areas of Physiology including bioelectricity, muscle contraction, cardiac and circulatory physiology, sensory physiology and toxicology. Two phases can be distinguished in Bernstein's career, each neatly exemplified by a monograph. Most of the first phase is summarized in his *Untersuchungen über den Erregungsvorgang in Nerven- und Muskelnsystem,* 1871 (5), (Investigations on the excitation process in nerve and muscle systems) while the results of the second phase are collected is his *Elektrobiologie* published in 1912 (6).

The first period belongs to the classical electrophysiology, in which he developed delicate stimulation and measuring instruments, examples of the high quality of work performed in the German mechanical workshops of the middle of the 19th century. To this period belongs his work on the velocity of propagation of the action current in nerve, the determination of the time course of the excitation wave and its relation to the demarcation current. All these investigations were carried out with the aid of the differential rheotome which he designed. Since the deflections of the galvanometer elicited by single nerve impulses were too small to allow the accurate recording and measurement of their time course, his device stimulated the nerve periodically 5 to 10 times per second. The stimulus contact and the contacts which periodically short-circuited the galvanometer were adjustable. Bernstein called his apparatus a rheotome because it cut out' certain pieces from the plane of a current curve, thus allowing the construction of the actual current curve from the values, a process not unlike modern averaging problems.

Two main conclusions were drawn from these investigations: a) The electrical changes are propagated at the same velocity as the wave of excitation. In other words, the conduction velocity of excitation in nerve, which Helmholtz measured by stimulating the nerve at two different points and recording the two different intervals between stimulation and muscle contraction, was identified with the velocity of propagation of an electrical waveform. In addition, Bernstein was able to measure, in 1882, the 'time loss in the nerve end organ' i.e. the neuromuscular synaptic delay, for which he gave a value of 0.3 ms. b) Each individual nerve impulse has a wave form of a certain duration. Bernstein measured the duration of the electrical wave and found it to be 0.7 ms although, as he pointed out, the properties of the ballistic galvanometer would tend to give low values. Later on, Ludimar Hermann introduced the term of 'action current' for this electrical wave.

Grundfest (1965) called attention to the fact that Bernstein had observed the overshoot of the nerve action potential as early as 1868. This finding was confirmed by Hermann in 1881, after initial negative results. This topic will be discussed in the next issue of this study. In Bernstein's diagram, included in his 1871 monograph, the amplitude of the action current is more than twice that of the demarcation current, or 'nerve current'. He writes (1871, p28): "In fact ... the magnitude of the negative deflection can become greater than the nerve current, and may even exceed it many times". However, this overshoot is not mentioned in his second book on *Elektrobiologie.* The reason for such omission is likely to be, as Grundfest (1965) suggested, the fact that there was no room for such observations in his new 'membrane theory of bioelectric currents' formulated in 1902 (7).

With this paper Bernstein opened a new era in biophysical research. He recognized that there was nothing more to be gained with the existing theories and methods and decided to take advantage of the new ideas that were being developed in a period of high creativity, in the fields of electrochemistry, molecular physics and thermodynamics. To do so, he first acquainted himself with these new developments - a task, remarkable at an age in which most investigators cease to look for new ways or fail to apply them fruitfully.

Bernstein's Membrane Theory

Based on a thorough survey of what was for him contemporary science, Bernstein proposed an ingenious hypothesis to explain both the resting properties of the excitable cells -nerve and muscle fibers- and the changes which occur in them when they become excited. Bernstein's new hypothesis rested on three main assumptions: Living cells are filled with a liquid, the cytoplasm, which is basically an electrolytic solution. This is surrounded by the cell surface membrane, permeable to some ions; while the potassium ions can diffuse out, the phosphate ions cannot. As a consequence of the above there is a permanent electrical potential difference across this membrane and is what we know as the resting potential. Finally, upon excitation, the permeability to ions increases and as a result the resting potential disappears or decreases to a very low value.

"A consequence of this theory would be that the negative deflection must achieve a maximum value which would be given by the magnitude of the membrane potential which during stimulation would not be able to reverse itself" (1912, p 105). As we see, there was no room in this theory for an overshoot. The original correct observation was the victim of the new theory. In his 1912 monograph Bernstein presented the theory of electrical processes in the organism in a modern fashion, still recognizable viewed from the end of the 20th century permeability was regarded correctly by Bernstein due to the presence of pores in the membrane which today we call channels. The resting potential would be according to him, due to the tendency of potassium ions to leave the cell. The value of this potential would be given by the Nernst equation, 1888 (8), 1889 (9). This paved the way for the future in recommending the use of the cathode ray tube as an indicating device.

The true explanation of electrotonic potentials was indicated by later experiments of Matteuci (1863) who found that electrotonic effects occurred if in place of the nerve he used a wire wrapped with thread soaked in a conductive solution. He thought that the spread of potential away from the electrode was the result of the 'diffusion of electrolysis'. This conclusion was strengthened by the observation that the spread was reduced by the use of amalgamated zinc sulfate wire, as was the electrolysis.

Iron-wired Models of Excitable Fibers

Hermann,1879 (10), 1899 (11), 1905 (12) explained the tendency of an electric current to spread along the nerve on the basis of a very simple observation: the spread of electric current along a metal wire immersed in a salt solution. According to this view, the nerve was thought to consist of a core and a sheaf. The core is a good conductor of electricity and the sheaf, or the boundary between the core and the sheaf is a poor conductor. He used a model (a central metal core surrounded by a moist sheaf formed by cotton and kaolin saturated by 0.6% NaCl) and arrived at the correct interpretation; that is, the polarization resistance between the wire and fluid was the cause of current spread. Another popular model was that of Hering (a liquid core surrounded by a moist envelope).

When an electric current is applied to a portion of a nerve between a pair of electrodes, the current spreads along the nerve by a mechanism analogous to that in a submarine cable. This behavior is referred to as the cable properties or core-conductors properties of the nerve.

Hermann (1879) was aware that the core-conductor properties alone did not explain the process of generation of action currents by the nerve. He argued, however, that a region of a nerve generating an action current will behave as a sink of current; consequently, there is a flow of current between the excited and resting zones of a nerve. He proposed that the local current mediated the spread of a physico-chemical process underlying the propagation of the nerve impulses. Hermann's theory received strong support when Ostwald pointed out the similarity, at least superficial, between the electrochemical processes that take place in an iron wire immersed in nitric acid and the conduction of the nerve impulse.

Working under the supervision of Ostwald, Heathcote (1907) published an extensive study of the properties of the passive iron wire; i.e. an iron wire covered with a layer of iron oxide formed by immersion of the wire in concentrated nitric acid. This iron-iron oxide wire corresponds to Hermann's core conductor. When a portion of the oxide layer is destroyed mechanically or reduced electrically, local currents are generated between the reduced (active) zone and the neighboring oxidized layer can be reduced by the continued spread of an 'wave of activation'. Much later, Lillie expanded these observations and confirmed the similarity between the passive iron wire and the nerve.

In 1872, H. Weber introduced the assumption that the radial currents in the core and the envelop fluid be ignored and calculated the distribution of current in the steady state (not time dependent) for such a model. The introduction of this assumption reduces the problem of the distribution of current and voltage for a cylinder from three dimensions to one. In addition, for there to be an appreciable spread of current the longitudinal resistance must not be too large compared with the radial resistance of the membrane. Therefore, this model is known as the 'linear core conductor model'.

The temporal aspects of the establishment of the current distribution were earlier thought to be related to the well known capacitative element observed in polarizable electrodes. It was on the basis of an analogy with polarizable electrodes, that the capacitative element was introduced in the equations of the linear core conductor model. In this form, the equation becomes identical with that for the flow of heat in one dimension as well as with the equation treated by Kelvin in 1855 in connection with the then new Atlantic submarine cables, (see Webster 1927,1955). However, the fact that the equations for the linear core model and for the submarine cables were identical was not recognized until the end of the century

(Hoorweg, 1898).

The general equation for a cable with longitudinal resistance and inductance, and parallel leakage and capacitance has, as particular examples in physics, the one dimensional equation for heat flow, the wave equation, the propagation of sound and electromagnetic waves, as well as the flow of electricity in conducting media. The role of cable properties of excitable fibers in the process of impulse conduction would not be fully understood until the 1930's.

C. Electrophysiology at the Turn of the Century

At the turn of the 20th Century research in electrophysiology was pursued vigorously in still another country: England. While in the middle of the 19th Century Germany had already the extremely productive School of Physiology founded by Johannes Müller, there was no physiological work being done in England.

In 1836 a chair of both Anatomy and Physiology was created at University College London. Its first holder, William Sharpey, was an anatomist. Physiology was taught from books and no physiological research was carried out. Nevertheless, one of Sharpey's students, Michael Foster [1836-1907] went on to found one of the most productive schools of physiology in Europe: that of Cambridge. Two of Foster's pupils were to achieve the highest recognition in neurophysiology: Sir Charles S. Sherrington [1857-1952] and John Langley [1852-1925]. Pupils of the latter included Keith Lucas, who died prematurely in an airplane accident in 1916, and E. D. Adrian later Lord Adrian.

Most of the work done until the 1930's was directed towards three main goals: a) Further characterization of the properties of the nerve impulse with the aid of improved instrumentation;b) Understanding the physiological function of the action of nerve fibers as carriers of information and c) The elucidation of the nature of the nerve impulse, of the physics and chemistry of excitation.

Properties of the action potentials

The basic experiment to show the existence of propagated disturbances in nerve was as puzzling to the early 20th century investigators as it had appeared to Galvani and Matteucci. If one pinches the end of a motor nerve still attached to its muscle (as in a sciatic-gastrocnemius preparation) the muscle enters into contraction, apparently simultaneously with the stimulus. "Nothing to be seen has happened in the nerve" -wrote the English physiologist Sir William Maddock Bayliss [1860-1924] in his classic treatise (Principles of General Physiology) in 1915 (13)-"yet, something must have passed along from the point at which the nerve was pinched, otherwise the muscle would have been unaware of anything having taken place at the other end of the nerve. It is usual to speak of a 'propagated disturbance' passing along the nerve or sometimes a nerve impulse. But, how are we to detect and investigate it in the nerve itself, apart from the indicating muscle?"

Time course. It was rather obvious that a point on a nerve in a state of excitation behaved as electrically negative to any other spot at rest; the electrical changes elicited by the stimulus at any point travel along the nerve making each point in turn electro-negative to the rest. This would explain the time course of the action potentials recorded with two electrodes (A and B). First, A will be negative and B positive; then A positive and B negative, thus giving rise to the typical diphasic deflection. But if electrode B is placed on a spot of nerve that has been 'killed', by heating it, for instance, the only electrical effect that will be seen is the negativity of A. As a consequence, the recorded action potential will now be monophasic.

All-or-nothing character of the action potential. The experiments of Keith Lucas (1909) (14) showed that the degrees of contraction of a muscle that can be produced by varying the strength of the stimulus applied to its nerve are not as numerous as the degrees of strength of the excitatory stimulus, but take place in a series of steps which are not more numerous than the number of motor nerve fibers supplying the muscle. This was taken as an indication that the varying degrees of contraction are due only to differences in the number of muscle fibers contracting. In other words, that each fiber, nervous or muscular, can only be excited to its maximal capacity or not at all. This fact had already been observed by Bowditch (1871) in directly stimulated heart muscle and the possibility that it could be applied to nerve was discussed by Gotch in 1902. However, the actual proof of the all-or-nothing character of excitation was given by Adrian in 1914 (15). By working on sciatic nerves that were anesthetized in certain segments, he was able to show that an action potential, after passing a region where its amplitude is decreased by the influence of the anesthetic, recovers its maximal amplitude when it enters a normal area.

Are there nerve action potentials of different kinds? This question attracted the attention of investigators. However, it could never be demonstrated that different stimuli give rise to more than one type of response in the nerve. Even though it had been observed that stimuli of long duration and slowly rising amplitude would produce abnormally long contractions of the muscle, this proved to be due to the fact that the stimulus was producing trains of action potentials and, consequently, repetitive, or tetanic stimulation of the muscle.

Specificity of the response elicited by nerves. The question had also been raised of whether the different effects obtained by stimulation of different types of nerves, were due to differences in their action potentials. The response was negative.

Refractory state. It was first shown by Gotch and Burch in 1899 (16), that if a stimulus applied to a nerve is followed by a second one at an interval of less than about 8 ms (depending on the temperature), the second one does not give rise to a propagated disturbance. This means that the nerve is inexcitable immediately after a state of excitation. Adrian and Lucas in 1912 (17), studied this phenomenon in detail using the muscle as an indicator. They showed that for the first 2.5 ms after the first stimulus the nerve cannot be excited by stimuli of any strength (absolute refractory period). From that time to about 12 ms excitability is lower than normal but increases gradually. This is the period of "relative" refractory state. Following this, until 28 ms, there is a period of increased excitability.

Fatigue. This question intrigued investigators of that period. Bayliss wrote: "If we regard fatigue as that result of activity by which a cell is less readily put into action again until a certain time for recovery has been allowed, it is clear that the refractory state itself is one of fatigue. Under ordinary conditions, however, the recovery is so rapid that it is impossible to demonstrate that a nerve is less excitable at the end of a long period of activity than at the beginning". Preoccupation with this question was related to the inability of the investigators to show the existence of active metabolism in nerve fibers, as demonstrated by oxygen consumption and the production of heat.

Bayliss adds: "... since we must suppose that the nerve is absolutely devoided of respiratory activity... the fact that this disturbance in normal nerve is propagated without diminution, suggests a physical process... energy is supplied to it as it travels. It is conceivable that the energy-giving material might require replacement by an oxidation process; but we are again met by the difficulty of the absence of heat production. A.V. Hill suggests that oxygen acts by keeping the machine in order, as it were, somewhat as oil in a motor does. It must be confessed that this seems to be a function rather unusual for oxygen to perform".

Summation and facilitation. Adrian and Lucas (1912) showed that there is a form of increased excitability which only occurs at the point of excitation. Indeed, if two inadequate (we should read subthreshold) stimuli are applied, the second following the first after about 0.8 ms, it sets up a propagated disturbance. It is clear that the first ineffective stimulus leaves behind it a change of some kind that persists for a measurable time and is added on to that produced by the second stimulus when this is applied. These observations foreshadowed the discovery of graded, subthereshold, non-propagated responses that Katz (18), following a similar technique, would demonstrate in the 1930's and Hodgkin would record directly, shortly after.

The compound action potential. Using for the first time the cathode ray oscillograph as a display or indicating device, Gasser and Erlanger (1922) (19), were able to distinguish in the action potential recorded from the frog whole sciatic nerve, a number of components or peaks, which they correlated with the spectrum of fibers of different diameters which make up the nerve. The action potentials generated by the individual fibers add up to the large compound action potential. The shape of this potential is smooth if the distance between stimulating and recording electrodes is short. However, as the distance increases the action potential is seen to separate into discrete peaks corresponding to fibers with different average conduction velocities. This can be compared to the runners in a race, who form a compact group but separate into subgroups as the distance increases due to their different speeds.

Physiological role of action potentials

As mentioned above, the electrophysiologists at the turn of the 20th century had already considered whether there was some feature in a nerve impulse that determined its effects at the nerve endings. It was clear, since the very beginning, that the main function of a nerve was that of carrying information or messages, and Langley had shown that the particular influence of a nerve on the effector organs which it innervates was not determined by the nerve trunk, but rather by the properties of the nerve terminals. Also, the question had been asked whether a nerve can conduct several types of propagated disturbances or action potentials and the response had been negative.

The main problem had been clearly formulated: what is the nature of the nerve code? How are messages transmitted along the nerves using just one type of physical signals? The answer to this question was provided by Adrian in a classic series of experiments performed on sensory nerves originating at various types of receptor organs. His results are summarized in a small but very fundamental book entitled "The Basis of Sensation: the action of sense organs". Soon after its publication in 1928

(20), this book became one of the cornerstones of modern neurophysiology.

Using a hybrid recording system provided with three stages of vacuum tube amplification and a capillary electrometer as a display device (with a total amplification of 1,700-1,800 fold) Adrian recorded the activity of sensory nerves of various types. He started with the same nerve-muscle preparation (sciatic-gastronemius) that Galvani and Matteuci had employed, and recorded from the sciatic nerve while the muscle was submitted to various degrees of stretch. While neither a killed nerve nor one attached to a slack muscle exhibited spontaneous electrical changes, he observed clearly how electrical activity appeared when the muscle was stretched. Many small waves, detected by the movement of the mercury meniscus of the electrometer, appeared. However, since the total number of fibers in the sciatic nerve is about 3,000 of which only a small portion are sensory fibers, the amplitude of the signals was too small to allow their analysis. For this reason, Adrian looked for a simpler preparation and he moved on to a very small muscle first described by Ramon y Cajal in his book *Textura del Sistema Nervioso* (Texture of the Nervous System) (1899) and later used by Keith Lucas in his experiments on the all-or-nothing properties of nerve and muscle fibers. This muscle, the sterno-cutaneous of the frog, is supplied by a nerve containing 15-17 fibers of which only one is sensory. This nerve was too short to record directly from it, but it joins a nerve trunk that contains about 500 to 1000 fibers. In collaboration with Zotterman, he cut small strips of the muscle until a single functional sense organ remained in the preparation. In this manner they could record a comparatively large signal, originating in a single fiber, when the muscle was stretched by attaching small weights to it. They discovered that the muscle spindle sends information to the central nervous system on the amount of tension applied to the muscle, in terms of the frequency of the action potentials generated at the sensory organ. In other words, Adrian found that nerves encode the information they transmit by modulating their frequency of discharge. This frequency increased as a function of the stimulus, and the nerve would fire first at a constant and later at a slowly decreasing rate.

Adrian soon realized that there was a fundamental difference between the sensory nerve organ terminal and the nerve itself. Indeed, while the direct stimulation of the sensory nerve fiber gave rise to only one or, with a large stimuli, two or three propagated disturbances, stimulation of the end organ with a constant stimulus resulted in a lasting discharge of the fiber. He formally described such a difference by saying that the nerve accommodates or adapts rapidly to a constant stimulus, whereas the end organ exhibits much lower accommodation and keeps firing action potentials for long periods of time. Adrian found that not all sense organs behave in the same manner, since other sense organs, particularly those which serve tactile sensations have a much higher degree of accommodation than the muscle sense organs. This is reasonable from a physiological viewpoint, as muscle tonus must be maintained continuously whereas it is an advantage to adapt rapidly to cutaneous sensations, such as those produced by clothing or the strap of a wrist watch.

So far, we have examined the main electrophysiological observations made in the first hundred years of this discipline. We should now enquire briefly into the interpretations of the phenomena described. In particular what were the ideas and hypotheses concerning the nature and origin of animal electricity?

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