A SHORT HISTORY OF ELECTROPHYSIOLOGY AND IT'S TECHNIQUES

Section III

Electrophysiological Instruments and Techniques.

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Recording instruments. Nowadays, to explain any electrophysiological event, we must think of both voltages and currents, as well as the constant that relates them (resistance or impedance). However, early workers in this field thought mainly in terms of the currents an excitable tissue can generate since this and the muscle contraction was all they could access in the pre-ohmic era. Today there are various instruments available to measure d.c. currents, whose names indicate their range of operation, from those which with the aid of appropriate shunts can handle huge currents, to the more sensitive ones, milliammeters and microammeters and the most sensitive, the galvanometers. Repeatedly in science the newer techniques and instruments are usually the less sensitive ones. The rapid and early growth of electrophysiology was made possible, however, by the invention and application of these sensitive instruments which measure the flow of electric current along a wire in the nanometer range and with a power of nanowatts. It would be interesting to write a history of galvanometers to illustrate the interactions between science and technology in the entire 19th Century but that would be far too cumbersome for this 'brief' history. To understand these instruments, their virtues as well as their shortcomings, and those of the devices that replaced them, a short digression is necessary. The block diagram shown in Fig. 1 represents a recording system typical of the middle of the 20th Century. The system consists of four main components: namely, a transducer (T), one or more amplifiers (A), a display unit (D) or indicator, and the biological signal (S_{in} and S_{out}).

A signal has been defined formally, as "a variation in the amplitude and/or the polarity of an observed physical quantity produced by a process whose mechanisms we desire to understand by experimental investigation" (Glaser and Ruchkin, 1976)(1). A recording system is one that converts a biological signal into an electrical trace that can be printed, stored and played back, such as an analog photograph of a cathode ray oscillograph monitor, or digitally in a computer memory. Each of the components of such a recording system has several properties which determine its performance and compatibility; that is, whether or not they can be connected together forming a functional chain in such a way that the output signal of the display or indicating device will be a faithful reproduction of the biological signal.

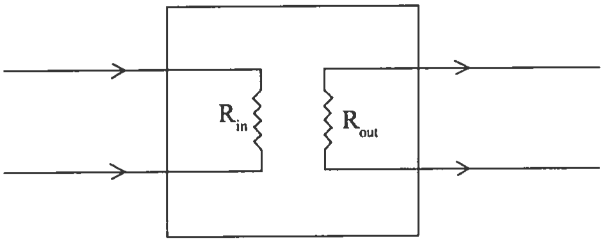

Figure 1a. Diagrammatic representation of one of the units of a recording and reproducing system. Each component has two wires that apply the input and two that carry the output. The resistors shown between the two wires of each do not represent real resistors, neatly soldered between the two wires, but the resistance "seen" by the incoming signals.

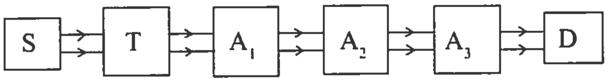

Figure 1b. A recording and reproducing system formed by connecting several of the above units in series (see text). It is important to realize that the input resistance is seen by the first amplifier. As a result the amplitude of the signal may be greatly reduced (see amplifiers).

The most important properties of each component are: a) the amplitude of the signal which can be fed into its input; b) the input and output resistance, or impedance; i.e. the resistance between the two input wires and the two output wires; c) the output resistance or impedance between the two output wires; d) the frequency response, or the range of frequencies which the component is

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capable of handling without distortion and e) the transfer function; i.e. the relationship between the input and output signals; for instance, an amplification factor of 100X.

The transducer is a device used primarily to change the energy of the signal from a mechanical, optical, or chemical signal to an electrical one. The biological signal is characterized, in turn, by its amplitude, by the spectrum of frequencies it contains, by its resistance or impedance and by its type of energy. Even if it is of an electrical nature, such as a voltage or a current, the source of the signal; i.e. the tissue, must be connected to the recording system by means of two wires, the leads or electrodes, the properties of which will become a part of, and determine the properties of the biological signal. If we want to assemble a recording system to study a given biological signal we shall have to select components (transducers, amplifiers and display units) which are adequate and compatible. For this we must follow two simple rules: the first is that each component should have an adequate bandwidth or frequency response to be able to handle without distortion, or loss, the signal generated by the previous component in the chain. The frequency response of each and all the components must be determined by the spectrum of frequencies contained in the signal. The second rule concerns the input and output resistances or impedances of each component. Two points must be kept in mind: a) If what we desire to transfer between each two adjacent components is mainly the time course, the input resistance of each unit should be as high as possible compared to the output resistance of the previous component in the chain. b) However, if our purpose is to transfer power or energy between the two components, the input and output resistances of each should be the same. For instance, in a sound-recording and reproducing system, the input impedance of the loudspeaker, expressed in ohms, should be equal to the output resistance of the amplifier to which it is connected.

With these notions in mind we can have a look at the galvanometer. This instrument, like an ordinary mercury-filled thermometer, can be regarded as a complete recording system in which the variable to be measured acts directly on a display component which also serves as a transducer. Galvanometers are instruments which detect the flow of electric current in a wire in virtue of the interaction of the electromagnetic field created by the current with another magnetic, or electromagnetic field. Indeed, they all depend upon the relative movement of a magnet and a wire, or coil of wire, through which an electric current flows. This current, we must keep in mind, is supplied directly by the tissue.

There are, basically, two types of galvanometer. Those in which the magnetic needle moves under the influence of magnetic field generated by the current flowing in a coil of wire. This is the "multiplier" that Schweigger invented, and Nobili improved by using two magnetic needles of equal intensity placed in opposite directions. The use of this double needle, called "astatic", first proposed by the French physicist Andre Marie Ampere [1775-1836], greatly increased the sensitivity of the galvanometer as it neutralizes the effects of the magnetic field of the earth. Similar in design were the galvanometers of Ruhmkorff and Bourbouze. Years later, the English physicist and engineer William Thompson, later Lord Kelvin [1824-1907], designed a greatly improved version of this type of galvanometer which became known as the "Kelvin type".

The second type, based also on a discovery made by Ampere, are galvanometers in which the magnet is stationary as in the D'Arsonval type and what moves is the wire carrying the current.(Fig. 2) In 1883 the mirror

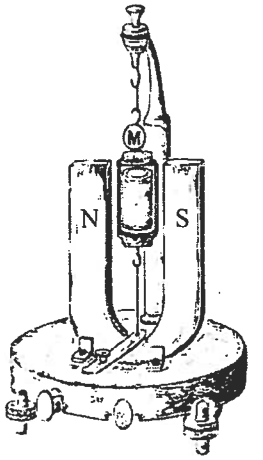

Figure 2a. Moving Coil Reflecting Galvanometer: used to measure currents from nerve. muscle and brain. Basis for nearly all present met

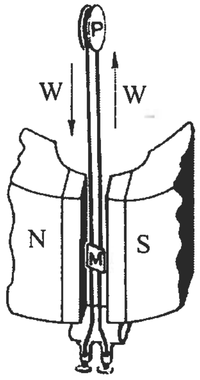

Figure 2b. Oscillograph: a refinement of D'Arsonval's Moving Coil Galvanometer.

galvanometer was invented by Lord Kelvin; a small mirror was attached to the wire carrying the current (Fig. 3). In this manner very small torsional movements of the wire could be mechanically amplified many times as the deflection of a beam of light directed to the mirror and reflected upon a scale.

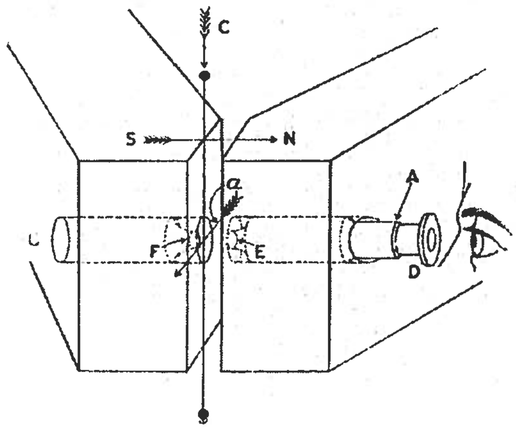

Figure 3a.

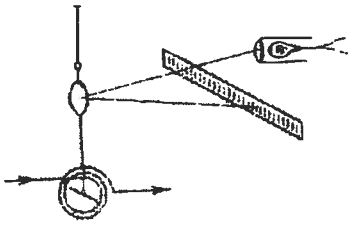

Figure 3b. Mirror Galvanometer

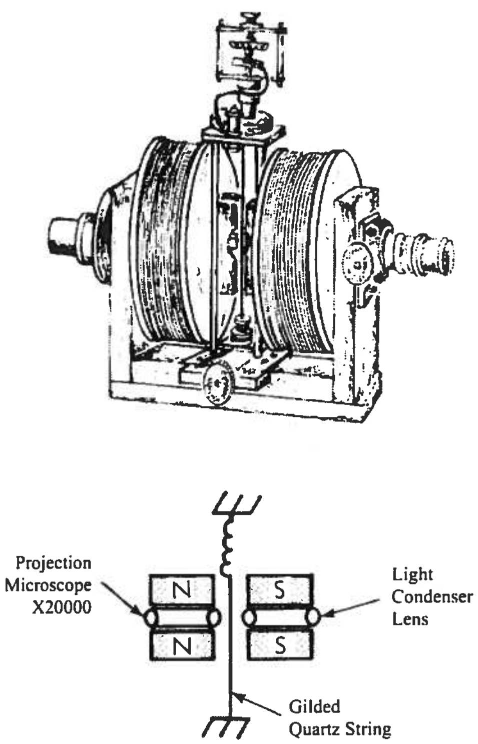

Figure 4. String Galvanometer: a further refinement of the D'Arsonval Galvanometer. Used by Berger to record alpha rhythm and EEG (1920s).

In the early 1920's a Dutch physiologist, Willem Einthoven [1860-1927], invented the string galvanometer, which consisted of a very fine wire stretched between the poles of a very powerful stationary electromagnet (Fig.4). When current flows through the wire this is deflected to one side or another depending upon its direction. The movements of the wire is magnified by a microscope and photographed by projection through a slit. This proved to be a very sensitive and useful combination; it made possible the recording of electrical activity generated by the heart (EKG) and brain (EEG), both of intact animals and human beings.(Fig.5) This was a remarkable achievement considering that the EKG signals have a power of only about 10^{-9} watts. Einthoven's string galvanometer was used by cardiologists until the middle of the 20th Century. However, the use of galvanometers in electrophysiological research has a number of serious limitations. The first is that the current flowing through the instrument must be supplied by the tissue being studied, and since what is expected from the instrument amounts to a transfer of energy (to move the magnetic needle or to induce a torsional movement in the wire), its sensitivity will be maximal when the resistance of the tissue, which acts as a source of current, is the same as the input resistance of the galvanometer. This is often difficult to achieve. A second drawback is the actual mass of the coil or moving piece. This introduces inertia in the system and results in a delay between the actual variable

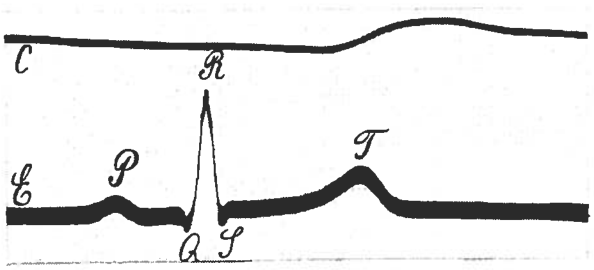

Figure 5. EKG Recorded by Einthoven Ca 1913

and recorded signal, thereby reducing the frequency response or bandwidth of the instrument.

In addition, the very movement of the wire in a magnetic field induces a current in it, of a direction opposite to that of the current which is being measured. This is known as 'damping'. Damping can be decreased by the use of vanes, moving in air or a viscous liquid. This combination of inertia and damping are responsible for the natural frequency of the galvanometer. In other words, since the moving system is brought back from its resting position by a force which induces another force of an opposite direction, oscillations will occur similar to those of a pendulum. This inherent periodicity makes it difficult to estimate the real value of the deflections being measured. Therefore, galvanometer designers aimed at making the instruments as completely aperiodic as possible. That is the reason why galvanometers are often referred to as "ballistic" devices.

The capillary electrometer. This is, like the galvanometer, a display instrument which receives its energy of operation directly from the tissue being studied (Fig.6). However, it is characterized by withdrawing very little current from the preparation and having much lower mass and inertia. Indeed, the capillary electrometer is practically aperiodic. Invented by the French physicist Gabriel Lippmann in 1878, this became the most important instrument in electrophysiological research during the last years of the 19th Century and the first quarter of the 20th Century. Basically it consists of a slightly conical capillary tube containing mercury, immersed in a 20% solution of sulphuric acid. The mercury is forced by pressure into the capillary until it enters into contact with the acid solution at a convenient point. One electrode is connected directly to the mercury also in contact with the acid. If the two electrodes are at different potentials, so that the mercury in the capillary is positive, its surface tension decreases and, as a result, it is forced down by its weight towards the tip of the capillary. If, conversely, the mercury in the capillary is made negative with respect to the acid, its surface tension increases and the meniscus retracts. The interphase acid-mercury behaves like a condenser of a very low capacity. Until the mercury comes to rest, a certain amount of current flows through the instrument. After this, no current passes. The movements of the meniscus are very rapid and completely aperiodic. The reason for the changes in the surface tension of the mercury are chemical. When the mercury in the capillary is made positive it oxidizes and becomes "dirty". When negative, it is reduced to bright metal. In the first instance the surface tension decreases and in the second it increases.

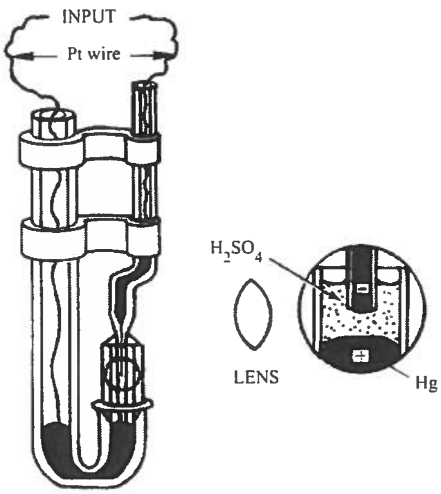

Figure 6. Capillary Electrometer: used for early measurements of nerve, muscle, EKG and EGG. Measured displacement by microscope and recorded on film.

The movements of the meniscus are photographed by projection through a microscope to a slit behind which a photographic plate is moved at a high speed. The curves obtained in this manner can be corrected for each instrument by recording square test pulses.

Stimulation devices. A variety of stimulation devices were used during the first century of electrophysiological research. They can be divided into three main groups: mechanical, chemical and electrical.

Mechanical techniques have only an anecdotal interest, since all of them act by injuring the nerve or muscle and their effects are, therefore, irreversible. Indeed, a simple way to stimulate a nerve is to cut it with a pair of scissors, but this has the disadvantage that upon repetitive stimulation the nerve becomes shorter and shorter. The same applies to all methods of mechanical stimulation. At the beginning of his studies Du Bois-Reymond used a small-toothed wheel which compressed the nerve against a hard surface eliciting an impulse each time the nerve was squeezed. A similar method used a small hammer. Another bizarre system consisted of letting drops of mercury fall on the nerve. The intensity of the stimulus was adjusted by changing the height from which the mercury dropped.

Chemical stimulation acts by damaging, or at least depolarizing, the nerve or muscle. It was achieved by the use of acids, bases and heavy metal ions. Salts were used, and those of potassium are of physiological interest, as they depolarize excitable cells by reducing the concentration gradient of the K ions across the surface membrane. Others of physiological interest include those compounds which bind calcium ions, such as oxalic acid and sodium citrate. In the absence of calcium the excitable membranes become unstable and fire impulses. Soon, all these methods were replaced by electrical stimulation. However, it must be recalled that the first action potentials recorded from the giant axons of the squid were elicited by applying a crystal of oxalic acid to the preparation, thus establishing their nervous nature (J. Z. Young, 1965).

Electrical stimulation has many advantages; it can be applied to a well-defined region of the tissue, its strength can be adjusted accurately and, if proper precautions are taken, it can be applied an indefinite number of times without causing permanent damage to the cells which are stimulated. All the techniques of electrical stimulation depend on the flow of current across the surface membrane of excitable cells by placing them in a voltage gradient. The main differences between the various techniques depend on the source of the electromotive force and the time course of the current flow.

Initially, nerves were stimulated by connecting them to the leads, or terminals of a voltaic battery. This was essentially what Galvani did with his bimetallic forceps (the galvani forceps, by the way, are still useful to check the functional condition of a nerve during dissection). Later on, the voltaic pile was replaced by new, more efficient batteries. The French physiologist Claude Bernard [1813-1878] favored the use of an odd-looking device, which consisted of several small voltaic piles arranged in series in the shape of a pair of forceps. The main disadvantage of the use of d.c. currents to stimulate is the fact that, if allowed to flow for long periods, they will cause electrochemical changes in the tissue; this could be prevented by keeping the pulses short.

A further step was represented by the use of a condenser discharge for stimulation. This was widely used around the turn of the century and for many years thereafter. The time course of the applied current can be accurately controlled by changing the resistance through which the condenser is discharged.

A problem faced by the early electrophysiologists was the need to apply two consecutive stimuli separated by a brief and adjustable time interval. Until this was made possible by electronic circuits using vacuum tubes and transistors, physiologists had to rely on mechanical contact breakers. The best known of these was the "Lucas pendulum" after its inventor, Keith Lucas. The main component of this device was a steel spring that could be bent and kept in this position by a stop; upon removal of the stop, the spring would swing back and its tip, following a semicircular path, made contact with two or more wires. The intervals between the closing of the different contacts could be adjusted in the millisecond range.

Electrophysiology in the first three decades of the XXth Century. At the turn of the century, research in electrophysiology was pursued vigorously in still another country: England. While in the middle of the 19th century Germany already had 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 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.

New recording techniques: vacuum tube amplifiers. A new era in electrophysiological instrumentation began in the 1880's with the discovery by the American inventor, Thomas Alva Edison [1847-1931], of the conduction of electric current in a vacuum; that is, within a glass bulb, similar to an ordinary light bulb from which the air has been pumped out. The current flowed from a filament made hot by an electric current to a plate made positive with reference to the filament by means of a high-tension battery (100-150 V). This device became known as a diode (Fig.7).

In addition, Edison made the far-reaching observation that the current flowed only in one direction, a phenomenon that did not receive adequate explanation until 1899, when J.J. Thompson [1856-1940] discovered the electron. It had been clear, as J.A. Fleming [1849-1945] had emphasized, that the current was due to something travelling from the hot filament to the plate, or anode, and after Thompson's discovery the suggestion became irresistible that the hot filament was actually emitting the unit particles of negative electricity which we now call 'electrons'.

In 1904 J.A. Fleming had the foresight to realize that the phenomenon of "thermoelectric emission" had commercial applications for the reception of wireless waves. Indeed the early radio experiments sought diligently for any device that would react to the minute alternating currents produced in a receiver of wireless waves (radio receiver or tuner). The frequencies involved were well beyond the power of any mechanically operated indicator. Fleming's valve, by allowing the flow of current

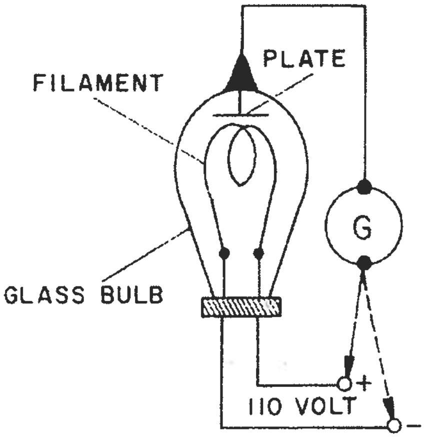

Figure 7. Diode vacuum tube developed by Thomas Alva Edison in 1883.

in only one direction, produced a d.c. or low frequency (audiofrequency) output from a radio frequency a.c. input. Therefore the d.c. current could be made to activate the diaphragm of a telephone receiver, producing a sound if the radio waves were modulated at an audible frequency (ca.2,000-20,000 Hz). Thus, Fleming's two-electrode valve became the most widely used detector of electromagnetic waves (the process of detection being dependent upon the rectification of the radiofrequency alternating currents set up by the incoming signal).

In 1907 Lee De Forest [1873- 1961] obtained a patent in the U.S.A. for a valve similar to that of Fleming but with a third electrode consisting of a grid of wires interposed between the filament and the anode (Fig.8). He found that the electrical potential of this grid exerted a remarkable control over the filament-to-anode current. For this reason the three-electrode valve, or triode, could be used as an amplifier as well as a detector. The triode was first used for electrophysiological purposes by Forbes in the U.S.A., and in Europe by Daly and Adrian. The latter described the new device in most enthusiastic terms in his book "The Basis of Sensation" (1928) (2). The following paragraphs, taken from Adrian's book, vividly describe the functioning of the first electronic amplifiers:

"Fortunately [wrote Adrian, in 1928] the detection of very small and rapid electric changes has recently become a problem not confined to physiology, and our difficulties can be solved by the use of methods devised for wireless communication...wireless telephony became possible only with the introduction of the three-electrode valve which was developed on a large scale during the war and is now an article as widely advertised as the motor car or the safety razor"

"The valve (triode) acts as an amplifier because it lives up to its name and does act in much the same way as a valve or tap on a water line. Very small forces expended in turning a tap will alter the rate of flow of water down a pipe, and so control the large forces which may be developed in the hydraulic machinery supplied by the pipe. By some such arrangement it would be possible to construct an amplifier which would 'receive' very small changes of mechanical force and would deliver a faithful reproduction of these changes magnified a thousand times or more."

"In a valve amplifier the output is a current derived from a high tension battery of 100 volts or so. This current is passed through the valve, where it is carried by a stream of electrons emitted by a filament heated by a low tension battery. The electron stream is made to flow through the meshes of a wire grid, and the whole operation of the valve lies in the fact that the current that passes through the meshes is altered by a small amount..... its potential is controlled by the small electric changes that are going to be amplified, and the result is that the current passed through the valve by the high tension battery is also controlled by those changes"

"In effect, small changes in potential in the input circuit cause large changes in the output circuit, and the latter follow the former instantaneously because there are no moving parts in the valve (other than the electrons) and therefore no inertia to be overcome. A single valve may produce a 50-fold amplification of the applied potential, and greater amplification is obtained by coupling several valves in series..... Valve amplifiers for detecting wireless signals were developed during the war and were applied to physiological research as soon as the war [WWI] was over, by Forbes in America, Daly in Britain and Hoeber in Germany."

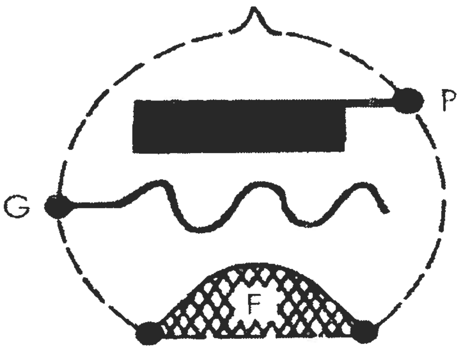

Figure 8. Triode, vacuum tube, or Audion, developed by Lee De Forest in 1907.

"We are no longer bound to use a sensitive recording instrument to detect the action currents in a nerve cell; all we need is one which reacts rapidly enough to follow the course of the currents without undue lag. At present the most perfect instrument as far as rapidity is concerned is the cathode ray which is deflected by applying a potential difference to two plates between which the stream passes. The possibility of using such a tube for recording action currents was suggested by Bernstein as long ago as 1912..... It has now been used with conspicuous success by Erlanger, Gasser and their co-workers for studying the time relations of action currents in the isolated nerve trunk, but there are still certain difficulties in obtaining a permanent record which limit their usefulness."(3,4)

The new amplifiers. The early vacuum tube amplifiers used in electrophysiology were of the type called a.c., or condenser-coupled, as the various stages of amplification were connected by condensers. These amplifiers could only be used for the study of changing signals, such as action potentials, but they were useless for the recording of steady potential levels, such as the resting potential.

It is interesting, when dealing with the electrophysiological instrumentation available in the 1930's to reproduce A.L. Hodgkin's reminiscences about that period (5). Hodgkin, then a young scientist, was to become one of the leading electrophysiologists of our time.

"I was lucky -wrote Hodgkin- because I inherited a Matthews oscilloscope and other electrical equipment from Grey Walter. In those days it wasn't considered proper to use an amplifier built by someone else. So I constructed a condenser-coupled triode amplifier in a series of biscuit tins which I painted bright blue. At that time there were no electric soldering irons, no resin-cored solder, and the valves, which were usually microphonic, needed antivibration mountings".

"In the late 30's-adds Hodgkin (Hodgkin et *al* 1977)- we were becoming 'professionals' and the objective of designing electronic equipment was not to make some neat miniaturized unit but to build up as massive and imposing an array of racks and panels as you could get- possibly with the idea of cowing your scientific opponents or dissuading your rivals from following in your footsteps. These large units were a nuisance if you wished to move to Plymouth or Woods Hole, but they did have the great advantage of being difficult to borrow when you were on holiday or writing-up results. In the end all equipment worked well though I had terrible and quite unnecessary trouble with it. At that time there was a sort of mystical idea that the noisiness of an amplifier varied inversely with the skill of the man who built it, and amplifier noise was regarded as a sort of moral penalty for bad workmanship."

Also in the 1930's the electrophysiologists realized that the recordings from high resistance sources offered particular problems which resulted in the attenuation of the signals.

Successful recording from high resistance preparations and signals picked up with microelectrodes only became possible when head stages of high input impedance were incorporated or added to the amplifiers.

The first high input impedance stages, which became known as cathode followers, were designed by Jan F. Tönnies [1902-1970], a German neurophysiologist and electrical engineer. Tönnies worked at the start of his career in Vögt's Brain Research Institute, in Berlin, where he designed the first ink-writer electroencephalograph and one of the first differential amplifiers. From 1936 to 1939 he worked at Gasser's laboratory at the Rockefeller Institute in New York City, and was there at the same time as Hodgkin in 1938 and used Tönnies' cathode followers in his work on the local subthreshold potentials in crustacean axons.

Hodgkin acknowledged Tönnies' contribution to electrophysiological techniques: "Then the whole business of shock artifacts was shrouded in mystery and I did not learn to think rationally on this subject until I was at the Rockefeller Institute in 1937. There I met Dr. Tönnies, who looked after the electronics in Gasser's group; he told me to forget about radiation fields and other irrelevant ideas... and to think only in terms of electrical leaks, stray capacitances and actual spread of current in the tissue....Tönnies pointed out that it was essential to use a cathode follower if one wished to make accurate recordings from a high resistance preparation like a single crab axon.".

As Offner (1984)(6) has emphasized, the field of bioelectric potentials rapidly developed during the 1920's and 1930's, with such complete cooperation of physicists, electrical engineers, and physiologists, that the dividing lines soon became blurred to the point of disappearance, the one-time technologists becoming physiologists and biophysicists , and the physiologists becoming among the most capable in the field of electronic instrumentation.

The first d.c. coupled amplifiers suitable for the study of resting potentials were not developed until the late 1930's, and those used for biological applications were all designed and constructed in the workshops of physiological laboratories. An example is the paper by F. Buchtal and J.O. Nilsen in 1936 (7). They described one such amplifier that had a baseline shift of only 100µU over a three-hour period. Another classic paper on d.c. amplifiers is that of P.O. Bishop and E.J. Harris (8) who discuss the limitations of direct-coupled valve amplifiers and offer a method for assessing their performance. They describe a multichannel high gain direct-coupled amplifier designed for use in biological applications requiring high input impedance.

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