

A VACUUM TUBE MICROVOLTMETER FOR THE MEASUREMENT OF BIOELECTRIC PHENOMENA*

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The formulation of the electrodynamic theory of life (Burr, '32, Burr and Northrop, '35) required that four questions be asked of nature.

1. Do living organisms possess steady state, or direct current, potential differences?

2. Can these potential differences be measured in such a way as to be free from the usual ambiguities of electrical measurement, i.e., can the determination of potential differences be made independently of resistance changes and current flow?

3. Do these potential differences reflect an unorganized chaos or are they related in such a way as to produce definable electrodynamic fields?

4. If such fields are present, are they merely by-products of the living process or are they determinants of the pattern of organization?

A little reflection will indicate that the answers to the above questions can be found only as a result of the development of a new technic. It is essential that a device be designed with which potential gradients can be determined independently of resistance changes in the system measured and without the introduction of the artefact of current drain from the system measured. Virtually none of the traditional instruments for direct current measurement satisfy these requirements. The few that do, present other technical features which make them extremely difficult to handle. In addition to the difficulties of measurement, there is the equally important consideration of the contact of the measuring instrument with the living system. The contact of any metal with living tissue introduces innumerable artefacts which cannot be controlled. Ideally, connection to the animal should be made through stable, reversible electrodes which introduce few or no artefacts into the measurement.

A network which fulfills the above conditions has been designed

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and tested under rigorous conditions during the last eighteen months. Using this, it has been possible to record electrically the following: (1) the instant of ovulation in the rabbit (Burr, Hill, and Allen, 1935), in the rabbit and cat (Hill and Greulich, 1936); strain differences in cancer bearing and cancer immune mice and potential changes antecedent to the gross appearance of cancer in mice (Burr, Smith, and Strong, 1936); (3) electrical concomitants of menstrual cycles in the human female (Burr and Musselman, unpublished); (4) changes in electrical potentials in the oestrous cycle of the white rat (Rogers, 1936); (5) potential gradients in the chick embryo (Burr and Hovland, unpublished); (6) potential differences in salamander embryos (Burr, unpublished).

And, finally, it has been possible to demonstrate in both chick and salamander that the potential gradients in the embryo produce electric fields which can be measured from one-quarter to one-half a millimeter outside of the living organism. In other words, using a technic the description of which follows, it has been possible to answer with reasonable certainty three of the original questions imposed by the electrodynamic theory.

Living organisms do possess direct current characteristics. These can be measured with accuracy sufficient to make possible mathematical treatment. The potential differences are not chaotic but produce organized electrical fields. However, it is yet to be determined whether these fields provide simply *another* map of organization or are prime factors in the determination of the pattern of organization.

The data which have been collected seem to indicate that with this device it is possible to record, in a very considerable number of circumstances, those electrical changes which are concomitant with alterations in physiological activity. Furthermore, the activities of intact living organisms can be studied with a minimum of interference. In other words, electrical records can be made of minute alterations in physiology. Under certain conditions anesthesia may be required, but the data at hand suggest that chlorotone or amytal, while they may lessen the magnitude of the polar differences, do not entirely wipe them out. This technic, therefore, should be a new and powerful weapon for the analysis of fundamental biological activity.

In order to meet the conditions outlined above, it was necessary

to design an instrument which would draw as small a current as possible from the system under test. That is to say, the measuring instrument must have as high an input impedance as possible. The classical instrument which fulfills this particular requirement is, of course, the quadrant electrometer in one of its various forms. This instrument is, however, one of the most difficult of all physical instruments to operate. Even highly skilled workers in physical laboratories have experienced so much trouble with it that it has been virtually abandoned as a research tool. This fact, coupled with the relatively low sensitivity of the instrument, is sufficient to rule it out completely as far as biological studies are concerned.

Therefore, attention was turned to commercial vacuum tubes which can readily be made to fulfill this last condition. In the last few years, a large number of circuits have been devised for the use of the FP-54 Pliotron or equivalent electrometer tubes. This tube seems unsuitable for the measurement of D.C. potentials in living systems. The reason for this lies in the fact that the mutual conductance of the tube has been greatly reduced in order to attain very high input impedance. To make the tube sensitive to small applied potentials, a very sensitive galvanometer must be used (of the order of 10,000 megohms sensitivity). At this sensitivity short time fluctuations become serious, and more troublesome still, the zero point continually drifts. Experience has shown that very high input impedance is not necessary and can be, in fact, a distinct liability. At very high input impedances the shielding requirements become very severe. An input impedance of 10 megohms is sufficient to handle even "microelectrodes" whose resistance may be several hundred thousand ohms.

The problem was one of creating an instrument which would fulfill the following rather extensive specifications:

- (a) The device shall have high input impedance, i.e., minimal current shall be drawn from the specimen under test.
- (b) The device shall be of high sensitivity. As a limit, a potential difference of 10 microvolts shall be measurable.
- (c) The device shall have high stability. Random fluctuations and general unsteadiness of the zero position shall be reduced to the lowest possible figure.

- (d) The device shall be widely independent of external electrical disturbances. The specimen under test shall not be "shielded."
- (e) Provision shall be made so that potential differences applied can be read off the instrument directly in microvolts or some multiple thereof.
- (f) The sensitivity of the device shall be independent within wide limits of the resistance of the specimen under test. This condition is, of course, bound up with condition (a) above.
- (g) The device shall be readily portable.
- (h) Standard radio parts shall be used in its construction as far as possible, to keep the cost at a low figure.

In the following description of the instrument as actually constructed, it will be seen that all these requirements have been fulfilled in a high degree.

As a starting point the circuit devised by Wynn-Williams⁸ was chosen and experiments initiated to determine how it might be modified to meet the requirements laid down. During the course of the work various ideas were incorporated but lack of space forbids a complete bibliography of all the works consulted. The general mathematical theory upon which the device was based is given by Wynn-Williams. Several designs were tested but the one finally adopted is shown schematically in Figure 3.

Of paramount importance was the type of radio tube to be used. After considerable study the power triode No. 112-A was chosen, the factors influencing this choice being (1) its large transconductance, (2) the fact that it is a non-heater type with relatively low temperature filament, (3) its low plate impedance. The reasons for the importance of these points will appear later.

The two tubes in Figure 3 form two arms of a Wheatstone network, the other two arms of which are ordinary ohmic resistors. Tube No. 1 receives the potential to be measured, tube No. 2 acts as a dummy, the function of which is to balance out the steady plate current of the input tube so that with no potential difference (p.d.) impressed on the first tube, no current flows through the galvanometer G. Upon impressing a p.d. on the first tube, the effective resistance of this arm of the network is changed proportionally and a deflection of the galvanometer results. The relation between the

galvanometer current i_g and the applied p.d. is given by Wynn-Williams as

$$i = \frac{\mu e}{2x + G \left(1 + \frac{x}{R}\right)}$$

wherein

i = galvanometer current
 μ = amplification factor of 112-A
 e = applied e.m.f. to be measured
 x = plate impedance 112-A
 G = galvanometer resistance
 R = bridge arm resistance

If, as is usually the case, $G \ll x$ then approximately

$$i = \frac{\mu e}{2x} = \frac{ge}{2} \text{ where } g \text{ is the transconductance of the first tube.}$$

While this analysis has been shown by several writers to be nothing more than a rough approximation, it is sufficient for the purposes herein described. It may be noted from the second expression that there is need for a large g if the device is to be sensitive.

A very important contribution to the theory of these devices was made by Wynn-Williams in the paper mentioned, when it was shown that it is possible to compensate automatically for the effect of filament battery variations. By operating the two tubes at slightly different filament voltages, a condition is reached whereby small fluctuations in the filament battery produce no variations in current through the galvanometer. This is important, since any storage battery shows slight variations in voltage which in turn would lead to unsteadiness in the instrument zero. The 7.5 ohm resistor R_4 , shown in Figure 3, is the means whereby this condition is realized in practice.

It may be shown that fluctuations in the "B" battery supply can also be to some extent compensated if the following conditions hold

$$\frac{X_1}{X_2} = \frac{R_1}{R_2}$$

where the subscripts 1 and 2 refer respectively to the first and second tubes and their associated bridge resistors. This is the reason for choosing 10,000 ohms as the value for the resistor arms since the dynamic plate impedance of the 112-A tube has approximately this value under the conditions of use in the bridge.

The comparatively low plate impedance of the 112-A tube is advantageous for our problem since, in general, the greater the impedance the greater will be the effect on the galvanometer of extraneous electrical disturbances. With this low value of plate impedance it has not been found necessary to shield the "A" or "B" batteries in any way, thereby greatly adding to the portability of the set.*

The circuit described so far does not differ essentially from that proposed by Wynn-Williams, but although it is of considerable value to the physicist for certain work, its value to the biologist would be virtually nil. The principal reason for this lies in the fact that in all commercial vacuum tubes a current flows in any external circuit connecting the grid and filament. This "grid-current" is independent, within considerable limits, of the resistance in the external circuit, and hence will cause potential differences across resistors in the grid circuit proportional to the value of these resistors. It is easy to see that if a specimen is connected across the input terminals, a fictitious p.d. will register on the galvanometer, which may, in point of fact, be many times larger than the true p.d. of the specimen under measurement. This, of course, would completely invalidate any results so obtained. In order to convert the Wynn-Williams bridge into a practical biological instrument, it is necessary to eliminate this spurious grid-current.

The method employed to eliminate the grid-current makes use of the well-known principle of "floating grid." It is known that if the grid of a vacuum tube, otherwise operating normally, is isolated from electrical contact with any other element of the tube, the grid will acquire a certain potential (floating grid potential). If, now, the grid is biased by means of a battery to precisely this potential, it is found that the grid-current is eliminated.

In order to bring this state of affairs about practically, a variable grid bias on the input tube is employed in conjunction with an amber-insulated switch, S1. The procedure is first to balance the set by means of the plate controls with S1 at position 2. S1 is then turned to position 3 and the set rebalanced with the grid controls. With a

* This condition arises because the dynamic input capacity of the tube depends on the magnitude of the voltage amplification. To minimize battery pick-up this capacity should be as small as possible. The 112-A, on account of its small μ and low plate impedance, fulfills this condition very well.

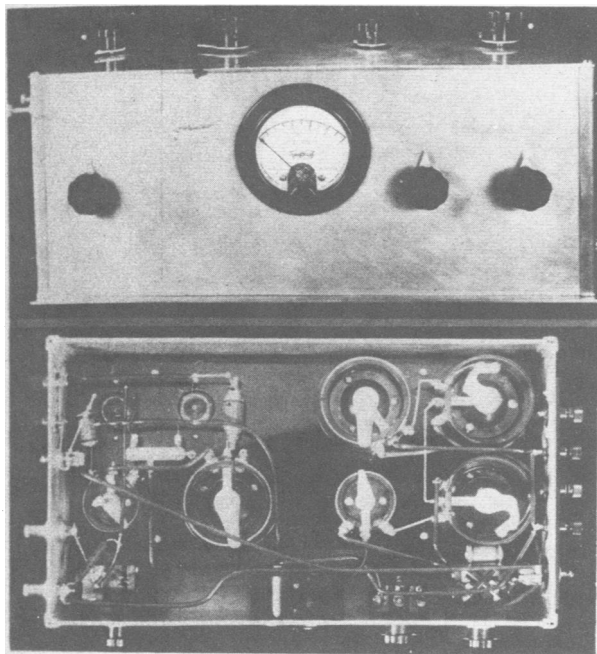


FIG. 1. Front view of the completed instrument.

FIG. 2. Top view with the cover removed to show arrangement of the parts.

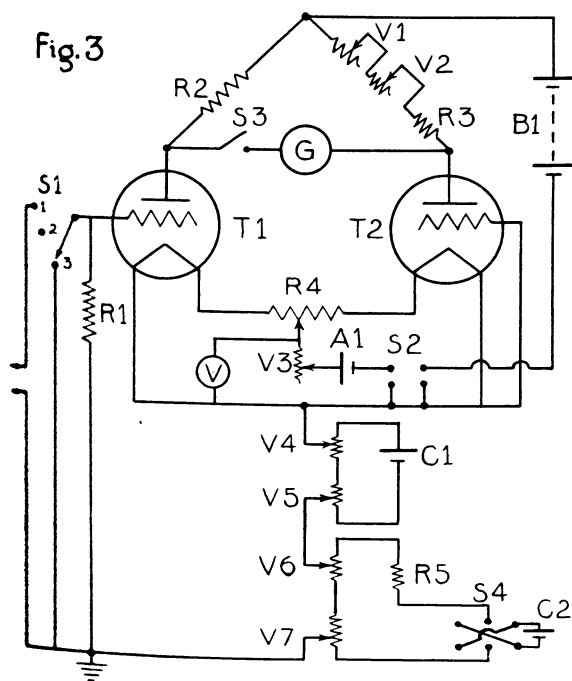


FIG. 3. Circuit diagram.

- A1—6 volt storage battery
- B1—45 volt heavy duty B battery.
- C1—3 volt C battery
- C2—1½ volt C battery
- G—Galvanometer
- R1—10 megohm grid leak
- R2—10,000 ohm "Electrad" fixed resistor
- R3—6000 ohm "Electrad" fixed resistor
- R4—7 5/10 ohm semi-variable resistor
- R5—100,000 ohm "Electrad" fixed resistor.
- S1—General Radio Type 339-B double pole, double throw switch with amber substituted for Bakelite insulation
- S2—Double pole single-throw toggle switch
- S3—Single pole single-throw toggle switch
- S4—General Radio Type 339-B, double pole, double-throw switch
- T1 and T2—RCA 112A radio tubes with bases removed.
- V—O-6 Voltmeter
- V1—General Radio variable resistor 20 ohms, type 214-A
- V2—General Radio variable resistor 2500 ohms, type 214-A
- V3—General Radio variable resistor 25 ohms, type 301
- V4—General Radio variable resistor 20,000 ohms, type 314-A
- V5—General Radio variable resistor 1,000 ohms, type 214-A
- V6—General Radio variable resistor 400 ohms, type 214-A
- V7—General Radio variable resistor 20 ohms, type 214-A

little practice, a point is quickly found where moving the switch to positions 1, 2, or 3 causes no change in the galvanometer. The grid current is now eliminated.

The value of the grid leak on the first tube was chosen to be 10 megohms. This figure was arrived at on the basis of a certain amount of compromise between specifications (a) and (d), given previously, since these two requirements are, to a certain extent, antagonistic. A very high value of this resistor would lead to correspondingly greatly increased sensitivity of the set to "electrical pick-up" and hence would necessitate elaborate shielding of the subject under test. The actual dynamic input impedance of the 112-A at floating grid is probably several times larger than this figure, but 10 megohms has proved itself to be a good value in practice. In order to minimize surface leaks, the tube bases were removed and the tube cavities filled with "ceresin" wax.

Included within the unit was a simple calibrated potentiometer so that unknown impressed p.d's could be measured directly in terms of a standard cell.

In the actual construction of the set only the highest grade wire-wound resistors were employed. All fixed resistors were "Electrad" precision resistors and all variable ones were General Radio Co. units. The only exception to this was the 10 megohm input resistor which was a carbon type supplied by the S. S. White Dental Manufacturing Co. The switch S1 was rebuilt using amber insulation. All connections were carefully soldered using rosin-core solder. The actual construction should be undertaken only by an experienced mechanic who is thoroughly familiar with radio set construction. When constructed in this way the set becomes a highly trustworthy instrument of very considerable ruggedness.

Once the set is built some preliminary adjustment and a "breaking-in" period must ensue. The filament voltage should be set at 4 volts and the set allowed to run continuously day and night for some 250 hours. At first the galvanometer will not stay balanced for any length of time; it will drift continually in one direction. This aging process eliminates the drift which after several hundred hours of operation will disappear entirely. Thereafter, the set may be switched on at any time and will attain stability within one minute.

After this breaking-in period is over, the next important step is to adjust the 7.5 ohm resistor, R4, for the "Wynn-Williams

balance." This operation is performed with the galvanometer at low sensitivity (about 10^{-6} amp/mm is sufficient). The variable tap of R4 is first set at the extreme left end and the galvanometer brought to zero with the plate controls V1 and V2. The filament voltage is now dropped to 3.8 volts and the resulting galvanometer deflection is read. Suppose it is $+X_1$. The voltage is now raised to 4.2 volts and the new deflection noted. It will be in the opposite direction, call it $-X_2$. Now the variable tap is moved to the extreme right end and with the filaments at 4.0 volts again balance

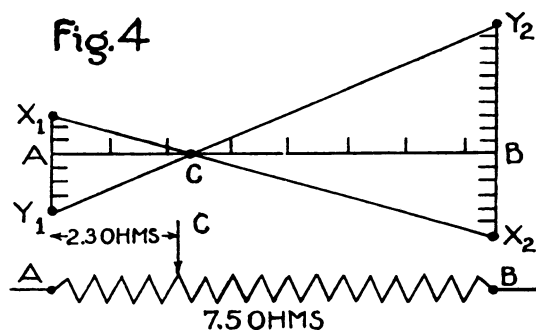


FIG. 4. Graph of galvanometer deflections indicating setting of the Wynn-Williams balance.

the set with the plate controls. Repeat the previous procedure with 3.8 and 4.2 volts and call the resulting galvanometer deflection $+Y_1$ and $-Y_2$ respectively. Draw a horizontal straight line on a sheet of paper 7.5 inches long representing 7.5 ohms (Figure 4, line AB). Then draw vertical lines at A and B representing to any convenient scale the quantities X_1 , X_2 , Y_1 , and Y_2 as shown. It will be found that the lines X_1Y_1 and X_2Y_2 intersect AB very nearly at a single point C. Now set the variable tap on the 7.5 ohm resistor at a point which corresponds to C (AC ohms from the left hand side). As a result, small variations in the filament voltage about 4.0 volts will produce but little motion of the galvanometer. This adjustment once completed need not be made again.

The final operation is to set the grid bias for floating grid. The procedure for this has already been described (page 70). For the first several hundred hours of operation this setting may show some tendency to drift, especially with the set operating at maximum

sensitivity. The setting should, therefore, be checked and re-adjusted periodically. After sufficient aging this effect, too, will practically disappear.

For highest sensitivity ($10 \mu\text{V}/\text{mm}$) a Leeds and Northrup Type R galvanometer with a current sensitivity of approximately 3×10^{-9} amp/mm, a period of 2.7 seconds, and a critical damping resistance of 10,000 ohms has proved satisfactory. For many biological measurements, however, a galvanometer 10 times less sensitive is sufficient, such as a Leeds and Northrup portable galvanometer with self-contained lamp and scale.

The instrument described, when properly constructed and adjusted, fulfills all the requirements laid down in the original specifications in the highest degree and has proved to be an exceedingly trustworthy and powerful tool for bioelectric research. Furthermore, by replacing the 10 megohm grid leak on the first tube by a 100 megohm grid leak, the instrument can be used with glass electrodes. With a table type galvanometer, the zero point is stable and there is a sensitivity of 100 microvolts per mm. of deflection. This sensitivity is maintained even though the resistance of the glass electrodes is as high as 100 megohms.

It is clear from the above that in order to evaluate the potential gradients of a living system, an electrical circuit must be established in which only potentials existing in the experimental material affect the measuring instrument. It seems impossible to measure the bioelectric potentials with any electrode in direct contact with living tissue, because an electrode, if reversible, has a potential conditioned by the concentration of a particular ion, or if not reversible, has a potential of uncertain or erratic magnitude. However, electrical contact can be made with a salt solution, if the salt be physiologically balanced, reducing to a minimum any potentials arising from the dissimilarity of the fluids at the point of contact. Or, if the salt solution be normal environment, the contact potentials are indeed a part of the total bioelectric potentials.

Of the known electrodes reversible to the ionic constituents of a Ringer's solution, only those reversible to the chloride ion have been developed to the high degree necessary for the present purpose. For the range of chloride ion concentrations found in solutions that are in physiological equilibrium, the silver chloride electrode is much more reproducible than is the earlier much used calomel electrode and can be used in the same solution that makes electrical

contact with the living system, thereby avoiding any liquid junction potentials. The silver-silver chloride electrodes, the preparation of which is described below, have been used in many exact electromotive

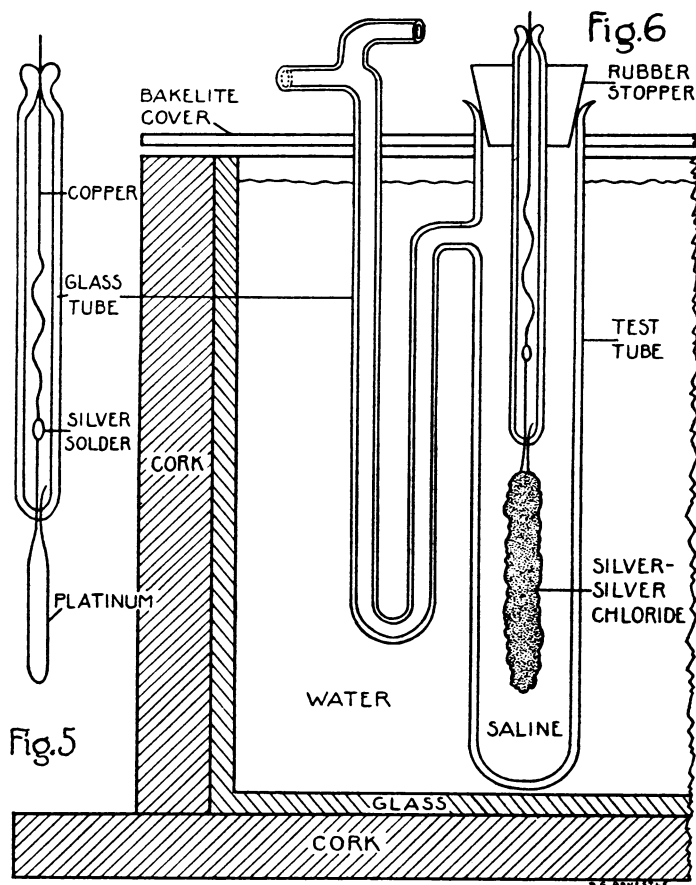


FIG. 5. Diagram of the electrode.

FIG. 6. Diagram of the electrode assembly.

force determinations by physical chemists and have been found to be stable and reproducible to within 10 microvolts or better when directly compared in the same solution. These electrodes were designated as Type II by Harned¹ and consist of silver obtained by heating silver oxide, supported on a platinum wire (Lewis²) and of

silver chloride formed by subsequent electrolysis in a hydrochloric acid solution.

To one liter of hot distilled water are slowly and concurrently added with vigorous stirring 200 cc. of a one molar solution of silver nitrate and 200 cc. of a one molar solution of sodium hydroxide. The precipitated silver oxide is washed by decantation twenty times, each time with its own volume of distilled water. This can be conveniently done in a tall graduated cylinder. The silver oxide is kept under distilled water until used, when the excess of water may be poured off. This quantity of silver oxide is sufficient for twenty or more electrodes.

A platinum wire which has been silver soldered to a copper wire is fused into a soft glass tube, so that the projecting platinum is in the form of a loop as in Figure 5. The projecting copper wire at the top may be sealed in the glass by carefully contracting the tube around the wire. Wet silver oxide is placed on the platinum loop, and the oxide is reduced to silver by supporting the electrodes in a crucible furnace or in any simple electrical heater in which a temperature of 450°C . can be obtained. The silver produced is in a very finely divided condition and appears white. Several successive applications of fresh oxide, followed by heating, suffice to form a cylinder of silver 5 to 8 mm. in diameter. Since the platinum wire need not be completely covered, the successive additions of silver oxide can be made without danger of cracking the glass-platinum seal.

The silver electrodes are chloridized for half an hour in a one-molar hydrochloric acid solution with the electrodes as the anode and a platinum wire as the cathode. A current of 20 m.a. per electrode is sufficient. The electrodes must be kept free of grease and need to be washed with at least four changes of the solution in which they are to be used, allowing them to stand for an hour in each wash. All solutions used, including the Ringer's solutions, should be made from chemically pure salts.

Figure 6 is a schematic drawing of one half of an electrode assembly to be used with micropipettes in conjunction with a micro-manipulator. Since the electrodes are sensitive to temperature, both electrode chambers are placed in the same small water bath, which has enough heat insulation to prevent rapid temperature fluctuations. The reservoirs are so connected that the micropipettes can be flushed out without disturbing in any way the solution around

the electrodes. Care must be taken to exclude all air bubbles from this system. Such an electrode assembly once set up will show less than 20 microvolts potential between the electrodes when clean pipettes are dipped into a saline solution.

This assembly can be modified to suit the needs of the investigator. Larger tubes in place of the micropipettes have been used directly on mice, rats, rabbits, dogs, cats, monkeys, and man, and tubes dipping into beakers of normal saline have been used for finger potentials in human beings. The important point in any assembly of electrodes is to keep the solutions surrounding the electrodes in as undisturbed a condition as possible.

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