ELECTRODE POTENTIALS AND POORLY POISED BIOLOGICAL SYSTEMS

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It was observed by Potter (1910) that during the decomposition of organic compounds by microorganisms, electrical energy was liberated which he measured by placing platinum electrodes into the inoculated and uninoculated portions of a medium separated by a porous partition. He did not interpret his results, however, as an expression of an oxidation-reduction system. It was not until 1920 that the true significance of Potter's observations was pointed out by Gillespie. As a result of studies on water-logged soils as well as pure cultures of bacteria, Gillespie introduced the idea of measuring the reducing intensity of bacterial cultures in terms of electrode potentials.

While reference has been made many times in the literature to the speed of reduction of dyes by bacterial systems, the importance of the "intensity" and "capacity" factors in this connection were not properly emphasized until the analysis of oxidation-reduction phenomena by W. M. Clark (1928) appeared. In order that we may deal with the present discussion in terms of the more modern interpretation of this subject, it may be useful to outline briefly the chief points of his analysis which bear directly on this work.

The accumulation of scientific evidence has so expanded the original significance of the terms "oxidation" and "reduction" that a meaning quite foreign to the original has evolved. It was considered formerly that the progressive addition of oxygen to products gave rise to higher and higher states of oxidation, whereas the loss of oxygen resulted in lower reduced states. All of which is true, but not comprehensive enough to include all reactions falling into these two categories mentioned.

It is known that reduction may be accomplished by the addition of hydrogen as in the case of the reduction of indigo to indigo white. The transformation of a ferrous chloride to a ferric chloride solution by the action of chlorine presents another point of departure from the original conception of oxidation. The ferrous iron ($\overset{++}{Fe}$) has lost two electrons as indicated by the two plus signs, while the ferric iron ($\overset{+++}{Fe}$) has lost three. Then in this transformation of ferrous to ferric iron by the activity of chlorine, the latter acts merely as the absorbent of an electron from the ferrous iron.

Oxidation then may not only be characterized by the addition of oxygen, the loss of hydrogen, but more especially by the loss of electrons. It is implied, of course, that the opposite is true in case of reduction. The reduction of one substance involves the oxidation of another, the former receiving electrons from the latter. Thus, the tendency of one system to reduce another is characterized by the readiness with which

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it gives up electrons to it. The intensity of this electron transfer may be expressed in terms of electrometric potentials. Of several systems which may be under consideration, the one which has the greater tendency to absorb electrons has an electropotential positive to the others and will oxidize any system with a potential more negative.

It is observed that an electrode of a noble metal (gold, platinum, or mercury) placed in a solution containing an oxidation-reduction system acquires an electron charge increasing with the reducing intensity of the system. The potential differences between this system and that of a potassium chloride calomel half-cell can be measured by connecting the two with a potentiometer and completing the circuit through a potassium chloride agar bridge.

The calomel half-cell is used as a standard of comparison in practical operations because of the ease with which it can be prepared and maintained at a constant value. The normal hydrogen electrode has been selected as the arbirtary zero electrode potential, but it is prepared and maintained at a constant value with difficulty. The normal hydrogen electrode is defined as a platinized platinum electrode held under one atmosphere of hydrogen and immersed in a solution normal with regard to hydrogen ions. The values of the saturated calomel half-cell, at various temperatures, have been determined experimentally, however, in terms of the hydrogen electrode and may be obtained from the tables of Clark (1928).

Electrode potentials are usually recorded as Eh readings, Eh = .150 volts at pH = 7.0 for example. The reading indicates that the difference between the potential of the system in question at pH = 7.0 and that of the normal hydrogen electrode is 150 millivolts, the former at pH = 7.0 being negative to the standard hydrogen electrode which, by definition, is pH = 0.

The necessity of stating the pH of the system at the time at which the reading is taken becomes obvious, since the Eh reading is affected also by a change in acidity. The Eh reading of ideal systems, such as the hydrogen and quinhydrone electrode, becomes .06 volts more positive progressively for each unit increase in hydrogen ions. Referring again to the reading above, the O-R potential of the system is not actually 150 millivolts negative to normal hydrogen electrode since the value of the latter is calculated at pH = 0, and that of the system to be measured is given at pH = 7.0. There are 7 units in pH variation between the two, which according to the figures above (7 x .06 volts) makes the system to be measured 420 millivolts more positive than it would appear if reduced to the same pH as the normal hydrogen electrode (pH = 0).

The change of .06 volts for each unit change in pH seems to hold for ideal systems, such as the hydrogen or quinhydrone electrodes, but it is subject to certain variations in complex biological systems. In sterile bacteriological media, however, pH changes can be prevented by buffers and the reducing intensity of the system in terms of potential-time changes easily noted. Even where pH changes occur, in such cases as bacterial fermentations, if these changes be followed, the potential-time changes offer some very valuable data for studying the electromotively active properties of the systems.

Poorly poised systems. A system is said to be poorly poised when it fails to resist a change in potential easily upon the addition of small amounts of electromotively active substances. This characteristic of O-R systems may be considered somewhat analogous to poorly buffered solutions in hydrogen-ion studies. The experiences of the writer indicate that many biological systems probably fall into this category. Poorly poised systems present certain difficulties in making electrometric potential measurements with a simple potentiometer circuit as will be discussed presently.

Vacuum-tube potentiometer circuit. The use of a simple potentiometer circuit has been found unsatisfactory for making O-R potential measurements in poorly poised systems due to polarization effects produced while finding balance. Particularly is this true of sterile bacteriological media. The magnitude of the current passing through the simple potentiometer circuit sets up a change of potential at the electrode, masks the potential produced by the medium itself, and leads to certain discrepancies in measuring the true potential of the medium in question.

Polarization effects may be avoided by the use of a vacuum-tube potentiometer circuit. A circuit of this type has been described by Allyn and Baldwin (1932) and will be referred to only briefly here. The principal feature of this circuit consists in reducing the magnitude of the current passing through the system to a very low value while making measurements. The system to be measured and the potentiometer are connected to the grid circuit of the vacuum-tube by a two-way switch. The galvanometer is placed in the plate circuit. After balancing the closed vacuum-tube circuit, the EMF to be measured and potentiometer are brought into the grid circuit by the two-way switch. This changes the grid bias which in turn changes the plate current, and a deflection is evidenced in the galvanometer. By giving the potentiometer a value equal to and opposite that of the EMF to be measured, the grid bias attains its original value; the plate current, likewise, becomes as before; and the galvanometer again reads zero. Hence, the reading on the potentiometer indicates the potential difference between the standard calomel half-cell and the system under investigation.

As stated above, the system to be measured is made a part of the grid circuit during the operation. The magnitude of the current in this circuit depends upon the negativity of the grid bias. The initial negative grid bias was so adjusted that the grid current had a very low value of approximately 10^{-10} amperes. This current had a very negligible polarizing effect on the electrodes in the systems studied.

Preparation of electrodes. There have been many references in the literature to the construction of electrodes, yet a beginner in the work encounters many difficulties that must be solved through experience. It would appear that the actual preparation of the electrode is of greater concern than the kind of regal metal used in its construction. The writer found no appreciable difference in electrodes made of gold or platinum. Discrepancies seemed to be caused, in the main, by fractures in the glass about the electrode or by improper cleaning.

The most satisfactory electrodes were obtained by sealing a small 22-gauge platinum wire in the end of a 5 to 6 mm. soft glass tubing.

The end of the glass tubing was held over a flame and glass permitted to flow in until the hole was almost closed, leaving barely enough room to accommodate the 22-gauge wire. The platinum wire was then inserted and sealed in firmly with a flame. An excess of glass about the seal was avoided. This type of electrode suspended in distilled water withstands autoclaving very well without cracking.

Jena glass was used also for sealing the platinum wire in the end of an ordinary soft glass tube and proved very successful, but required more time and skill than the simpler method just described. Pyrex tubing was investigated also, but the difference in the coefficient of expansion between it and the platinum rendered this glass less desirable than the ordinary soft glass tubing, especially when steam sterilization is employed.

Great care should be exercised in the cleaning of the electrodes, if comparable readings are to be obtained. The practice of suspending the

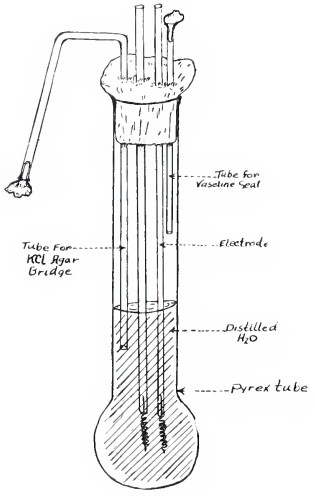

Fig. 1.

electrodes in hot chromic-sulphuric acid for some 30 minutes, rinsing thoroughly in distilled water, and flaming in an alcoholic flame, proved very satisfactory.

Some discrepancies were noted in the initial potential readings of electrodes in the same system depending upon the method of sterilization. Electrodes autoclaved while suspended by a cotton plug in empty Pyrex tubes, gave readings some 50 millivolts negative to those sterilized under water. This difference can hardly be attributed to microscopic fractures in the electrodes sterilized in steam. It was possible to have perfect checks between electrodes sterilized in this manner. Further, the difference between electrodes sterilized both in steam and under water was overcome and the electrodes brought into close agreement by intensifying the oxidation-reduction properties of the system by the addition of an oxidizing or reducing agent. It would occur that the surface of the electrodes suffers slight modifications, depending upon the method of sterilization and the modification is reflected by the initial readings of the

electrodes held in rather inactive oxidation-reduction systems. This difference was overcome after a time also by media placed under a vaseline seal. Greater consistency was obtained, however, with electrodes sterilized under water and it is believed that this practice is to be recommended.

Set-up for potential readings in sterile bacteriological media. The set-up depicted in Figure 1 was found eminently practicable for making O-R measurements of sterile bacteriological media. Large test tubes (1 x 8 inches) were heated and blown out to a uniform size at the base to prevent the electrodes from making contact with the glass. It will be noted that two electrodes, a tube for the KCl agar bridge, and a tube for the vaseline seal are firmly fixed in a cotton plug and set in the tube for autoclaving. The apparatus was cooled after sterilization, the KCl agar bridge filled with saturated KCl agar, and the barrels of the electrode with metallic mercury for making circuit contact. One end of a small rubber tube was fitted over the end of the tube for the KCl agar bridge and the other end held in the mouth. In the meantime, the KCl agar was prepared in a deep test tube and held in readiness aseptically at about 55° C. The whole apparatus was lifted from the tube in which it was autoclaved, the tube for the KCl agar lifted at a slight angle, inserted into the KCl agar, and filled by suction on the rubber tube. The end of the rubber tube was clamped momentarily to permit the agar to jell and the apparatus returned to the pyre tube in which it was sterilized.

The barrels of the electrodes were then filled with mercury for making circuit contact by means of long capillary pipettes. It would naturally follow that the system to be investigated has been prepared in a vessel of similar size and construction to that employed in sterilizing the electrodes in order to accommodate a complete transfer of the apparatus just described for O-R potential studies.

If it be desired to follow the potential time changes of the medium under a vaseline seal, the moisture may be driven from the sides of the tube with a mild flame and sterile warm vaseline introduced to the depth desired over the medium with a pipette through the tube in the cotton plug inserted for this purpose.

If aerobic or anaerobic fermentations be desired, the medium may be inoculated just before the introduction of the electrodes and handled accordingly. The writer has prepared several hundred systems as described here with no appreciable difficulty with outside contamination.

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