THE APPARENT OXIDATION-REDUCTION POTENTIALS OF BRIGHT PLATINUM ELECTRODES IN SYNTHETIC MEDIA CULTURES OF BACTERIA

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The study of oxidation-reduction potential changes in culture of various organisms has been pursued by many investigators since Potter (1911) first noted that the electrode potential of an inoculated medium was more negative than a sterile control. The extensive literature which has ensued contains results of determinations obtained almost entirely by the use of complex media of unknown composition. A few workers (Elema, *et al.*, 1934, Allyn and Baldwin, 1932, Thorne and Walker, 1936) have used media of known chemical composition as a substrate for the growth of soil and related organisms in redox studies. Thus far, however, to the writer's knowledge no attempt has been made to make similar studies with pathogenic or potentially pathogenic bacteria. As a result of the recent work on growth accessory substances or vitamin requirements (Koser, *et al.*, 1935, 1936; Sahyun, *et al.*, 1936; Knight, 1937 and Mueller, 1937a, b) it is possible to make potential measurements of growing synthetic-medium cultures of these latter forms.

The potentiometric method has been found superior to the colorimetric method for measuring potentials of this type. Preliminary determinations made with a conventional type K potentiometer bridge have shown such an arrangement to be entirely unsatisfactory for measurements in nutrient broth and synthetic media. The range of values obtained by consecutive readings was too wide to allow significance to be attached to them. This undoubtedly was due, in part, to polarization phenomena. A vacuum tube null-point instrument was con-

structed which eliminated polarization effects and gave identical consecutive readings in poorly poised systems. Despite this, however, discrepancies between duplicate electrodes and identical culture vessels were still encountered in both nutrient broth and synthetic media cultures. These irregularities appeared to be of sufficient magnitude and importance to warrant further study. From theoretical considerations there were several possible factors that might be involved: (1) Since a certain amount of stratification is known to occur in the growth of organisms in liquid media it seemed of value to investigate the effect of agitation of the medium. (2) In view of somewhat conflicting statements in the literature regarding electrodes, several types were used. (3) The poorly poised condition of simple culture media solutions suggested that appropriate poising substances might be tested. (4) The control of oxygen tension is obviously of importance both because of interference with hydrogen transfer and because of an oxygen electrode effect. In the present paper are reported comparative data obtained in a study of the relation of these factors to the apparent oxidation-reduction potentials produced in broth and synthetic media. Although it is recognized that blank platinum electrodes may in the presence of hydrogen give a hydrogen electrode effect, this aspect of the problem was not investigated directly since the test organism, *Escherichia coli*, ordinarily liberates a certain amount of this gas in its growth processes.

<div align="center">METHODS</div>

The vacuum tube circuit[1] is shown in figure 1. The vacuum tube heater voltage is set to approximately four volts by means of the 20-ohm variable resistance. Adjustment is made in the plate current line by manipulation of the potentiometers inserted therein so that no deflection of the galvanometer occurs when the tap key *A* is closed. Approximately 0.1 milliampere is read on *M*. During this period of primary balance the control grid is isolated and floating and as a result acquires a potential value

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[1] This circuit is a slight modification of one suggested by Dr. Otto H. A. Schmitt of Washington University, St. Louis.

at which the grid current is practically eliminated. The maximum grid current at any time was 10^{-11} ampere. Finally this grid is biased to this latter value by closing the special air-insulated switch B, closing C and adjusting the 6000-ohm and 600-ohm potentiometer resistances. The point of balance is again detected by using the key A. Switch B is now opened and the unknown half cell is connected to one pole of X and the saturated calomel fiducial cell to the other pole. The former cell is connected to the latter by means of an agar potassium-chloride bridge using a saturated potassium-chloride liquid junction. The
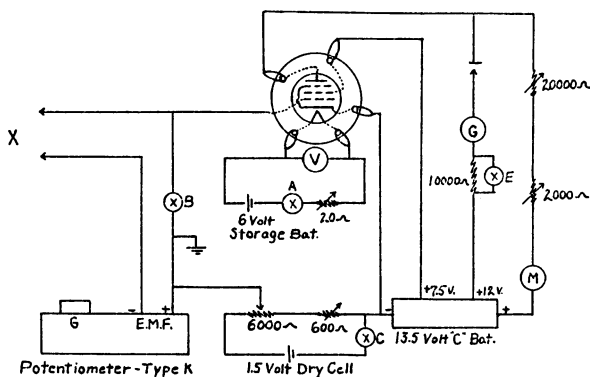

FIG. 1. VACUUM TUBE CIRCUIT

M, 0-1 milliammeter; V, 0-6 voltmeter; G, galvanometer; X, unknown half cell and reference half cell; B, air insulated switch; A, C, and E are simple toggle switches. Type 38 R. C. A. tube.

potential of the complete cell is then obtained as a direct reading on the potentiometer when G again shows no deflection. The calomel cell was prepared from electrolytic calomel, recrystallized potassium chloride and redistilled mercury. On checking against various buffers it was found to have the value 0.2364 volt at 37.5°C. A Leeds and Northrup lamp and scale type galvanometer having a current sensitivity of 0.025 microampere was used in most of the experiments. The performance of this instrument was checked against the more sensitive reflecting type having a sensitivity of 0.0005 microampere. The results were identical in all cases.

The electrodes were connected with the measuring apparatus through small mercury-containing steel cups embedded in high-melting-point paraffin. This material offered a suitable means of insulation because of its high resistance. Long, rectangular blocks of paraffin containing these cups were supported by the upper edges of the constant temperature bath in which the culture cells were incubated at 37.5 ±0.02°C. The culture vessels were 180 ml. electrolytic beakers without pours. These were closed with alkali-treated rubber stoppers containing five holes, two for electrodes, one for the agar potassium chloride bridge, one to allow for filling with medium, and a central one for the stirring arrangement. The latter was sealed either with mercury or oil. Mercury appeared to be more efficient and was easier to clean out than the oil; so it was used in the majority of the agitation experiments.

Electrodes were prepared by sealing into soft glass tubing of the desired size. Before the seal was made, a piece of 28-gauge platinum wire was silver-soldered to the part of the electrode entering the tube so that this small gauge wire could be brought out the other end and the glass sealed about it. This gave a glass tube sealed at both ends carrying the contact wire to which, on the outer end, a small piece of copper wire was silver-soldered for making contact with a mercury cup. An electrode of this type was easy to clean and handle.

For cleaning the electrodes, hot chromic acid, nitric acid and *aqua regia* were tested. As far as could be detected no one method was superior. The nitric acid method has been used in practice because the action of *aqua regia* is somewhat vigorous on fine gauze or foil electrodes. Those cleaned by heating gave no better results than were secured by the other methods.

The stirring mechanism, of the mechanical type, was operated by a ratio motor with output of 86 r.p.m. From this, by a series of pulleys and belts, the stirrers were operated in from one to ten cells simultaneously at a speed of approximately 130 r.p.m.

All synthetic media were prepared by weighing the requisite C.P. or Reagent chemicals on an analytical balance and adding to sterile re-distilled water. The water-clear solution was then filtered through a Seitz pad or Berkefeld N candle and incubated

to test for sterility. The basic synthetic medium, a slight modification of that of Koser and Saunders (1935), was composed of Na_2HPO_4 1.4 grams, KH_2PO_4 1.0 gram, NaCl 2.0 grams, $MgSO_4$ (anhy.) 0.1 gram, l-asparagine 3.0 grams, l-tryptophane 0.2 gram, d-glucose 2.0 grams and re-distilled water 1 liter. The final pH was 6.9. Standard sodium hydroxide was used to adjust the pH when additions such as cysteine hydrochloride were made. The nutrient broth used was composed of beef extract (Swift) 3.0 grams, peptone (Difco) 10.0 grams, NaCl 5.0 grams and re-distilled water 500 ml. This was adjusted to a pH of 6.9, sterilized in the autoclave, and to it added 500 ml. of sterile phosphate buffer (pH 6.9 at 37.5°C.). The buffer was present in a final concentration of m/30.

The culture vessels containing only the electrodes were sterilized by autoclaving. Before the electrodes dried, the medium, in 100 ml. quantities, was pipetted into the vessels. The agar potassium-chloride bridges were filled aseptically, inserted into the bung, and held in place by a section of close-fitting rubber tubing.

In the anaerobic experiments, commercial water-pumped cylinder nitrogen was purified by bubbling through sodium hydroxide solution and concentrated sulfuric acid, then run through a heavy wall pyrex combustion tube held in an electric furnace. The combustion tube was packed with small-gauge copper oxide wire and the latter was reduced in a stream of hydrogen before each experiment. Precautions were taken to blow all hydrogen out of the combustion tube before the culture vessels were connected. From the combustion tube the purified gas was led through a trap and then into a Y tube extending across and above the two rows of culture vessels. The nitrogen was bubbled through the medium in each vessel at approximately an equal rate. To accomplish this a piece of small-bore glass tubing was run through the center of each rubber bung and connected, using heavy-wall rubber tubing, to another piece of glass tubing of the same bore which projected at right angles from the nitrogen line. The two ends of glass tubing were approximated so that the chance of oxygen diffusing through the connection was lessened. Both pieces of tubing were stoppered

with a small piece of non-absorbent cotton. It is realized that these conditions do not yield absolute anaerobiosis, but it was felt that they were sufficient for the desired studies.

In every experiment all vessels were checked microscopically and culturally for contamination at the end of each determination. Counts were also made at the conclusion of an experiment to determine the total number of bacteria per ml. The vessels were labeled alphabetically in order to prevent confusion when taking measurements.

A carefully checked stock strain of *Escherichia coli* which did not ferment sucrose was used in all determinations. Inoculations were made directly into the medium from a twenty-four-hour agar slant culture. One hundred ml. aliquots were then pipetted out and, thus, each inoculated culture vessel had practically an equal number of organisms. However, no further attempt was made to standardize the number of inoculated organisms for it was thought that if good agreement was obtained consistently in individual experiments, the duplication of experimental results should not be difficult.

Every electrode was examined for flaws at the conclusion of each experiment and all were checked against one another by measurement of dilute solutions of ferrous-ferric salt mixtures. If defects were noted the results for the corresponding vessel were discarded from the data.

All experiments were performed at least three times. In each determination an average of ten separate culture vessels were used, each of which contained duplicate electrodes.

The hydrogen ion concentrations of the various media were determined before and at the end of the incubation period by means of a glass electrode.

<div align="center">EXPERIMENTAL RESULTS</div>

<div align="center">*Stationary synthetic medium cultures*</div>

Under conditions wherein no agitation of the substrate occurred, the experimental results were at variance between vessels containing identical electrodes, medium, and number of inoculated organisms. In figure 2 typical time-potential curves ob-

tained by using platinum wire spiral electrodes are shown.   The
double-line curve appearing under each letter represents a plot-
ting of values for individual identical duplicate electrodes in the
same culture vessel.   Representatives were chosen from neither
extreme, being selected from a group of ten vessels all of which
were incubated at the same time.   Other identical experiments
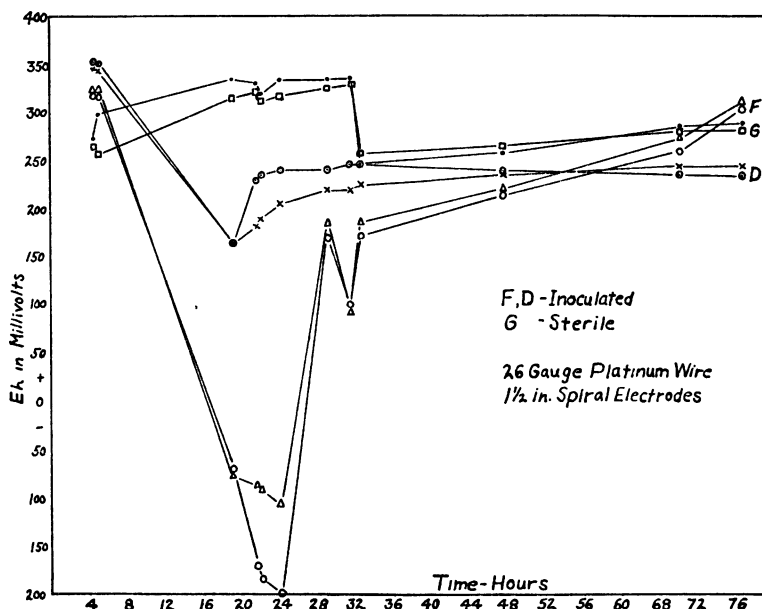always gave similar variations.   The maximum difference shown,

FIG. 2. TIME-POTENTIAL CURVES OBTAINED IN THE BASIC SYNTHETIC MEDIUM
UNDER STATIONARY CONDITIONS
Each curve represents values for a single electrode

which is approximately 444 millivolts, exists between values ob-
tained in separate vessels while the maximum between duplicate
electrodes in any one vessel is 95 millivolts.   In the majority of
cases, readings became more nearly concurrent after the culture
had been incubated thirty-six hours.   There was no indication,
when growth comparisons were made, that these or subsequent
irregularities were due to differential growth in various vessels.
  Results obtained by using other forms of platinum electrodes

showed a shift in the general oxidation-reduction intensity which will be discussed later, but discrepancies were still apparent.

Intermittently agitated synthetic medium cultures

Since the irregularities shown in the previous experiment are not readily explicable on the basis of mechanical defects or
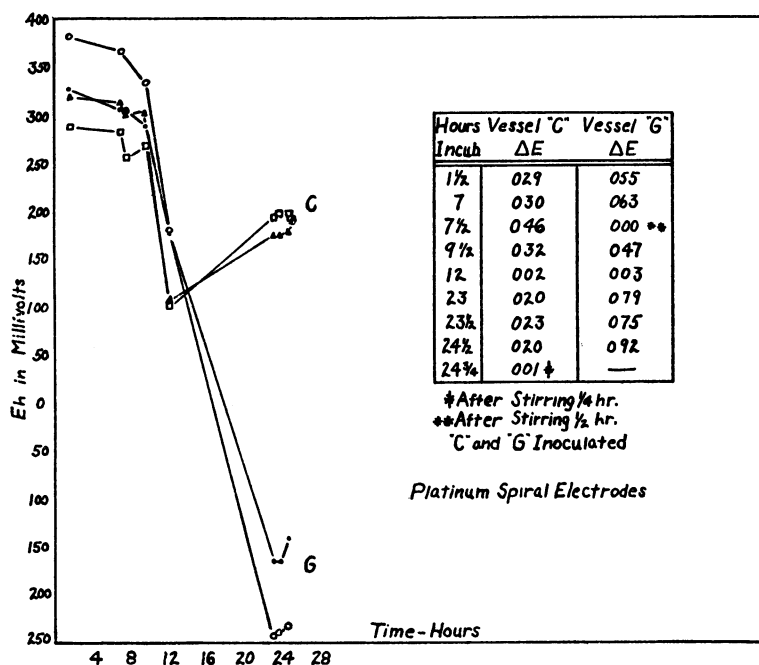

Hours Incub	Vessel "C" ΔE	Vessel "G" ΔE
1½	029	055
7	030	063
7½	046	000 **
9½	032	047
12	002	003
23	020	079
23½	023	075
24½	020	092
24¾	001 ✦	—

✦After Stirring ¼ hr.
**After Stirring ½ hr.
"C" and "G" Inoculated

Platinum Spiral Electrodes

Fig. 3. Effect of Agitation on Time-Potential Values Obtained in the Basic Synthetic Medium

ΔE, in millivolts, is equal to the difference between duplicate electrodes in the same vessel.   Each curve represents values for a single electrode.

methods used in cleaning the electrodes, the possibility is to be considered that localized growth, collection of gas bubbles, or temporary poisoning of one electrode might account for some questionable potential readings.

The plotted values in figure 3 are typical for cultures stirred for several short intervals during incubation.   ΔE, in millivolts, is equal to the difference between duplicate electrodes in the

same culture vessel at any one time. After seven hours' incubation under stationary conditions the electrodes, of the platinum wire spiral type, in vessel G gave a ΔE value of 63 millivolts. As a result of agitating for one-half hour ΔE was equal to zero. Likewise in vessel C the ΔE value was changed from twenty millivolts at twenty-four and one-half hours to one millivolt after stirring for fifteen minutes.

Better agreement, not necessarily absolute, was obtained between like duplicate electrodes in the same vessel under conditions of agitation, although there still remained a considerable difference among individual vessels containing the same medium. This again could not be accounted for by differences in growth or electrode defects.

Platinum electrode types in synthetic medium cultures under conditions of agitation

During the course of these experiments, various types of electrodes—platinum wire pin-point and spiral forms—as well as platinum foil and gauze types were tested. The time-potential relationships obtained in the basic synthetic medium plus 0.10 gram of cysteine hydrochloride are presented in figure 4. The curves in this figure were plotted from averages derived from duplicate electrodes in each vessel. In many cases ΔE values were rather large, so the mean is of questionable significance. These ΔE values ranged from 100 millivolts to zero millivolt, with approximately the same degree of irregularity appearing in both sterile and inoculated vessels.

The high values given by foil electrodes in the sterile medium, as shown by curves E and D, are typical for these and for gauze types. The potential in the same sterile medium was approximately 200 millivolts higher when determined by means of foil electrodes in contrast with readings with wire electrodes. The regularity of E and D as shown in the graph was not always as pronounced. In the inoculated medium, the wire electrodes and foil electrodes did not give the marked differences that were obtained in the sterile medium.

There was no agitation of the cultures between the twelfth

hour and the twenty-ninth hour. This results in the appearance
of a trough in the curve at this time. In other experiments
where cultures were not agitated or were constantly agitated
throughout the entire period, the greatest reducing intensity
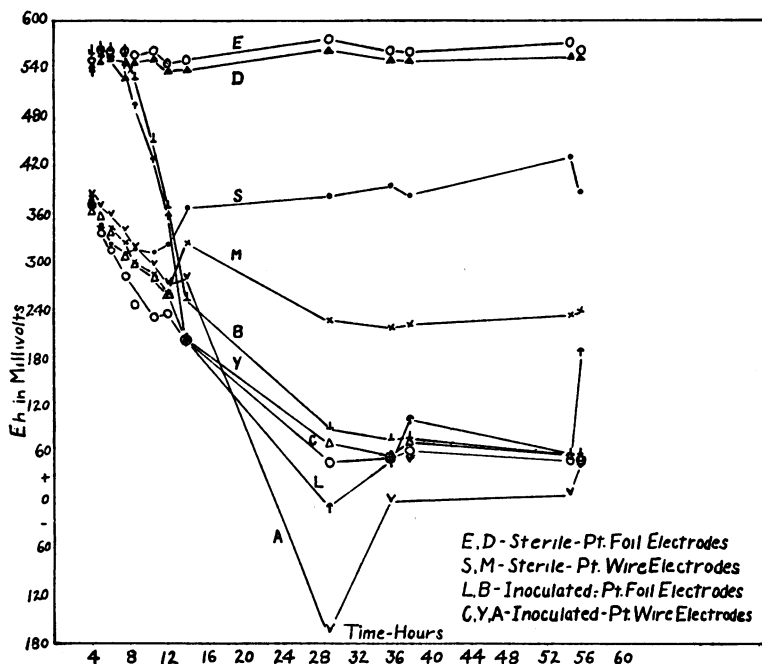appeared earlier. The inoculated vessels, again, regardless of

FIG. 4. TIME-POTENTIAL RELATIONSHIPS OBTAINED IN SYNTHETIC MEDIUM BY
PLATINUM FOIL AND WIRE SPIRAL ELECTRODE TYPES UNDER CONDITIONS
OF AGITATION

Each curve represents an average of the readings obtained from duplicate
electrodes in the same vessel.

the electrode type, showed an approaching similarity of values
after thirty-six hours' incubation.

Since the presence of cysteine in the medium might be con-
sidered to be a cause of the irregular values, the experiment was
repeated using the basic synthetic medium without cysteine.
Similar results were obtained. In the experiment shown in
figure 4 and in additional experiments of a similar nature, there

was no case in which any one electrode type appeared to give more consistent results than the other types.

Nutrient broth cultures compared with synthetic medium cultures under conditions of agitation

Previous workers, using broth and more complex media of unknown composition, have reported close agreement between duplicate electrode and duplicate vessel readings. The nutrient broth medium was used in these experiments in order to compare the results obtained in it with those in a synthetic medium.

The data obtained by using broth medium were consistently in better agreement. The majority of the ΔE values were in a range of 0 to 5 millivolts, with an occasional higher value of 20 millivolts. The difference between culture vessels was of the order of 5 to 10 millivolts. At times, apparently when metabolic changes were quite rapid, this difference was in a few instances somewhat greater. The results here, in contrast to those obtained in experiments using synthetic media, were fairly consistent throughout the entire period of incubation.

Semi-poised synthetic medium cultures

In the data thus far presented there is a suggestion that the irregularities still prevalent in a synthetic medium culture are due either to polarization of electrodes or to the absence of sufficient oxidizing-reducing material to give stable true values in the presence of oxygen. Polarization of the electrodes due to the measuring apparatus was ruled out because the irregular values were reproducible over a period of time and in different experiments. The electrodes in question, when checked against an inorganic oxidation-reduction system, always gave extremely regular results independent of type, shape or size of the metal surface. These discrepancies appeared, then, to be due to a culture medium poorly poised in the presence of air. Consequently, a reversible inorganic oxidation-reduction system was added to the synthetic medium in concentrations small enough so as not to alter the growth of the organisms perceptibly. Preliminary results, obtained by growing the test bacterium in the

synthetic medium and adding to the vessels (after irregularities were noted), small concentrations of potassium ferricyanide, were encouraging.

In figure 5 are recorded results obtained by allowing the organism to grow in the synthetic medium containing a 0.00031 M concentration of $K_3Fe(CN)_6$. Growth was excellent, and the
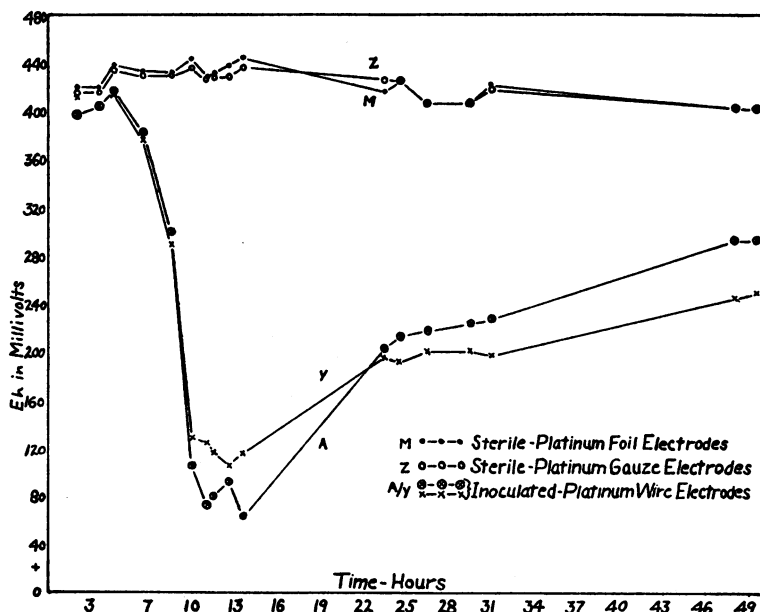

FIG. 5. TIME-POTENTIAL RELATIONSHIPS OBTAINED IN THE BASIC SYNTHETIC MEDIUM PLUS 0.00031 M CONCENTRATION OF $K_3Fe(CN)_6$ UNDER CONSTANT AGITATION

Each curve represents an average of the readings obtained from duplicate electrodes in the same vessel.

results were regular for the first ten hours of incubation. Such variations as did occur were of a small magnitude. In the sterile medium ΔE values were of the order of 2 millivolts, while in the inoculated medium the maximum variation was greater (20 millivolts). Experiments in which the concentration of $K_3Fe(CN)_6$ was increased gave almost perfect data. However, growth in such cases was retarded to a slight extent. It should

be noted that this poising of the medium dispelled differences in electrode types.

Synthetic medium anaerobic cultures

In view of certain peculiarities in the electrode effects previously noted, it seemed likely that some irregularities could be
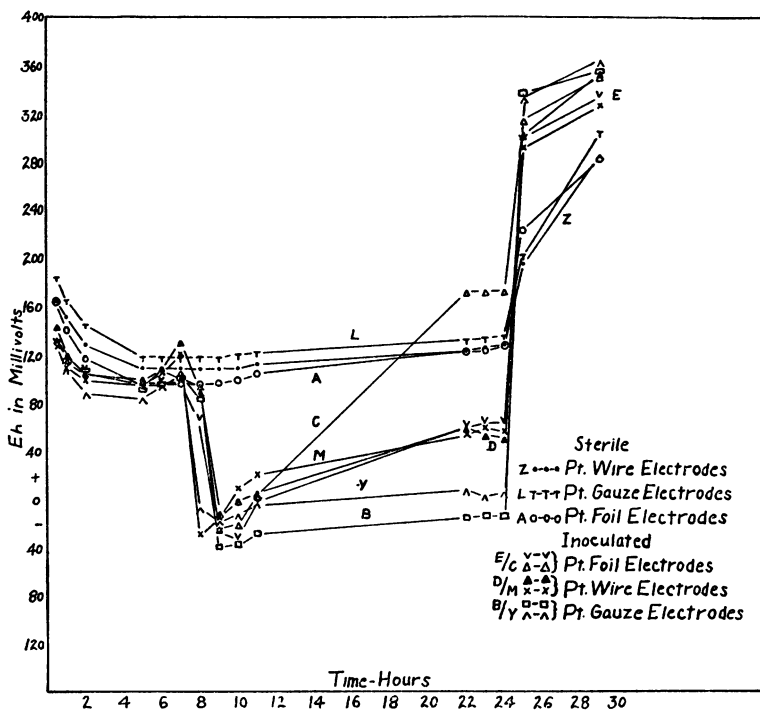

FIG. 6. TIME-POTENTIAL CURVES OBTAINED FROM THE BASIC SYNTHETIC MEDIUM UNDER ANAEROBIC CONDITIONS

Each curve represents an average of the readings obtained from duplicate electrodes in the same vessel. Pure oxygen was bubbled through the cultures beginning with the twenty-four hour period.

eliminated by removing atmospheric oxygen from the culture medium. The basic synthetic medium was chilled before being put into the culture vessels so that most of the oxygen could be removed by the stream of nitrogen before the temperature rose to a point where growth would become vigorous. One hour was

allowed for temperature equilibration. This was ample as judged by a control vessel. Time was counted from the end of this period.

Results obtained are plotted in figure 6 and ΔE values are given in table 1. It is to be noted, in contrast to aerobic determinations, that electrode values were regular for the first seven hours. After this time a rather sudden drop toward negative

TABLE 1

ΔE values in millivolts for basic synthetic medium anaerobic cultures

HOURS	STERILE			INOCULATED					
	Z	A	L	M	D	C	E	Y	B
½	1	3	10	1	2	3	2	2	2
1	2	2	6	3	5	3	2	0	2
2	2	2	4	2	2	2	1	0	1
5	3	2	1	0	2	0	0	0	1
6	2	2	1	0	1	0	1	0	1
7	2	2	1	1	2	0	0	0	0
8	2	1	1	4	7	1	0	12	7
9	2	1	0	6	11	1	4	7	19
10	1	2	1	10	29	1	7	3	39
11	2	0	0	11	34	2	10	2	56
22	1	0	0	10	40	1	9	29	71
23	2	0	0	2	40	1	10	25	70
24	1	0	0	5	43	2	9	28	78
25	19	0	3	0	11	8	0	26	22
29	11	7	17	3	35	1	0	25	21

potentials occurred. This latter phenomenon was practically constant as to time and rapidity of appearance.

The sharp rise to positive values occuring at twenty-four hours (fig. 6) was due to pure oxygen being bubbled through the cultures at this time. This introduced irregularities, especially in the sterile medium, as is shown by table 1.

DISCUSSION

The majority of workers who have studied redox potential changes in bacterial cultures have used broth or more complex

media. Using synthetic media it has been difficult to obtain such reliable data as could be obtained in a broth medium.

The use of synthetic media offers numerous possibilities for clarifying growth-potential relationships; but at the same time, many difficulties present themselves. The data show many of these irregularities and methods by which they may be circumvented.

In a non-agitated synthetic medium culture it is shown that anything resembling concurring values can only be obtained following thirty-six or more hours of incubation. After this time very little growth, in comparison to the earlier hours, occurs, so the measurements are not of what actually happened during the period of active proliferation but are of the end result of the entire process. As to whether the first portion of the time potential curve is of importance there is some disagreement. Under aerobic conditions of growth the point where the curve begins to become parallel with the abscissa axis would be dependent upon the number of organisms in the inoculum, upon the amount of poising agent present in the medium (in most cases this has been an unknown and disregarded factor), upon the oxygen tension of the medium and upon the reducing capacity of the bacterial redox systems. Any negligence in the consideration of these first three factors would make all points on the curve quantitatively erroneous. Where anaerobic conditions have been maintained, the effect of an important factor, oxygen, has been removed.

Constant mechanical agitation, under aerobic conditions of growth, is shown to be of some importance in obtaining better agreement of results. This must be the consequence of better equilibration due to the removal of poisoning material and zone effects. The observations suggested that there should be a tendency for electrodes to become sluggish due to coating with cells, etc., especially in old cultures with no agitation. It is recognized, of course, that agitation increases the oxygen tension in the medium with consequently more positive values. It is, likewise, realized that under conditions of agitation the metab-

olism of an organism may vary in certain respects from what would be termed normal, for it has been reported that agitation tends to decrease cell size after the early logarithmic period of growth (Mudge and Smith, 1933).

In a poorly-poised medium various types of platinum electrodes gave widely different results under identical conditions. There was no great deviation when these same types were used in a poised medium and none when tested in a simple inorganic redox system. Platinum electrodes were used almost exclusively, although a few gold ones were tried from time to time with no resulting obvious advantage. There was no apparent sluggishness of platinum types as compared with gold forms. In regard to this latter point Elema *et al.* (1934), Longsworth and MacInnes (1936) and others have reported the gold electrode to be more sensitive than platinum. This probably depends to some extent upon the type, as well as the amount, of electromotively active materials present in solution. An oxygen electrode effect would seem to account for the difference in values obtained with platinum electrode types.

It is shown that in order to obtain regular values during the growth of organisms in synthetic media under aerobic conditions, some oxidation-reduction system must be introduced which will act as an intermediate between the organisms or their products and the electrodes. This introduces a problem of some complexity for the proper substance must be chosen according to its E'_0 value and its toxicity. The amount added is also of prime importance since an excess may easily poise the potential in one region and thus give no indication as to what is actually happening as the organisms grow. Any one mediator can be good for only a limited Eh range. In regard to the latter point Kluyver and Hoogerheide (1936) have suggested the use of a mixture of oxidation-reduction dyes. There are many who would question the use of dyes and other compounds from the standpoint of toxicity but if an organism can be shown to be unaffected in growth or morphology by such substance there should be no valid objection. Undoubtedly the better values that can be obtained aerobically by the use of nutrient broth media owe their

superiority to the presence of one or more unknown substances that are acting in the same position as these known redox materials.

Anaerobically, in a poorly poised medium, very good agreement was obtained during the first seven hours of growth. At the end of this period there were large numbers of organisms as judged by turbidity. There was then a sudden tendency toward negative values and irregular results were again obtained. The rapid change in potential may be the result of a rapid pH change as suggested by Gillespie and Rettger (1936) but it is hardly reasonable to believe that such a change would occur almost instantaneously. Longsworth and MacInnes (1936) have noted that shifts occurred even when the pH was kept constant, but in no case were they as rapid as those noted here. The electrode phenomena encountered at this time are reproducible with regard to time and are apparently the result of changes occurring so rapidly that the inert electrodes are unable to establish an equilibrium. The almost concordant values, regardless of electrode types, obtained in sterile control culture vessels offer direct evidence against oxygen as an interfering agent in aerobic determinations of this type.

The fact that this bacterial oxidation-reduction system is oxygen labile (and certainly many, if not all, such bacterial systems would fall into this category), introduces another very important aspect into its measurement. How can it be said that a given organism establishes a characteristic potential in any medium if those systems responsible for the potential are exposed to the air previous to, and at the time of, measurement? The value obtained may stand the test of repetition by the initial investigator but it certainly has no absolute significance since it is dependent upon a number of factors over which no control has been exerted. An anaerobic or closed system would seem to be of prime importance.

pH changes have been thought by some investigators to be the source of some irregularities. The end pH value in the broth medium was in the alkaline range of pH 7.2 and that of the synthetic medium was in the acid range of pH 4.5. This latter

value was obtained in both poorly poised and semi-poised media. Hence, it is difficult to reconcile the irregularities as being due solely to pH changes during growth. There is, as yet, a lack of sufficient knowledge of the oxidation-reduction systems involved to warrant an attempt at making corrections for pH changes.

Many factors undoubtedly play important rôles in time-potential relationships of bacterial cultures. The data presented in this paper bring forth certain neglected aspects of the problems involved in the measurement of the apparent oxidation-reduction potentials established by bacteria when growing in synthetic media.

SUMMARY

In this study it is shown that a number of factors, neglected by other workers, are of importance in the measurement of apparent oxidation-reduction potentials in bacterial cultures.

The accuracy of results obtained from broth and synthetic medium cultures of *Escherichia coli* are compared. It is demonstrated that more comparable results are obtained in the broth medium. This fact is apparently due to the inherent presence of poising materials in the broth medium for when such materials are added to the synthetic medium, comparable results are likewise obtained.

The necessity for controlling the accessibility of oxygen to the culture medium is considered to be a factor influencing the magnitude of time-potential values. This is true because many if not all these redox systems are oxygen labile. Oxygen would, in such cases, affect the ratio, Ox./Red., upon which the potential determinations are dependent.

It is shown that zone effects, which are considered to result from temporary poisoning of the electrodes with bacteria or gas bubbles, account for slight variations. Agitation of the culture medium obviates these difficulties.

The type of bright platinum electrode used is only a factor for consideration when determinations in poorly poised media

are made in the presence of air. Under such conditions oxygen electrode effects are probably encountered.

The author wishes to express his gratitude to Drs. Stewart A. Koser and Dan H. Campbell for their interest and helpful criticism during the course of the work and in the preparation of the manuscript.

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