LXXVI. OXIDATION-REDUCTION POTENTIALS OF CULTURES OF *C. DIPHTHERIAE*. I.

By LESLIE FRANK HEWITT.

From the Belmont Laboratories (L.C.C.), Sutton, Surrey.

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Many bacteria, particularly facultative anaerobes, may be cultivated under widely different environmental conditions. It seems probable that the varying degrees of access to atmospheric oxygen of cultures must affect the metabolism of organisms and their general biological behaviour.

The bleaching of methylene blue is a familiar example of the reducing conditions developed in bacterial cultures during growth, but the disadvantages and uncertainties attaching to the use of dyes previously mentioned [Hewitt, 1930] necessitate the use of a more accurate method of following oxidation-reduction processes in bacterial cultures.

Reducing conditions established during growth may be followed, therefore, by the fall in potential of bacterial cultures. This has been observed by Gillespie [1921], Cannan, Cohen and Clark [1926] and Coulter and Isaacs [1929]. Fildes [1929] found that a limiting reducing potential was necessary for the germination of tetanus spores. In a previous communication [Hewitt, 1930] it has been shown that conditions of oxygen supply have a very marked effect on the potentials developed in cultures of haemolytic streptococci.

C. diphtheriae may be cultivated on the surface of solid media with free access to oxygen, in liquid media with a poorer supply of oxygen, or even anaerobically. The conditions governing toxin production are not understood. The organisms may show good growth but yield very little toxin. It is possible that such functions may be dependent on oxidation-reduction conditions in the medium, and this communication deals with observations of electrode potentials developed in cultures of *C. diphtheriae* under different conditions, with a view to possible correlation with the biological functions of the organisms.

Methods.

The apparatus and methods used were the same as those previously described in the study of haemolytic streptococci [Hewitt, 1930].

8 cc. of culture medium were introduced into each cell, the inoculum was a small loopful of the surface pellicle of a 24-hour broth culture of Park-Williams 8 strain of *C. diphtheriae* and the incubator temperature was 37°.

Results.

Peptone infusion broth (Fig. 1). Horse-flesh infusion broth containing 2 % of Difco peptone was used. The potential fell to $E_h = -0{\cdot}14$ v. during the first 10 hours' incubation and then more slowly, reaching a minimum value of just below $-0{\cdot}20$ v. in 75 hours. Even after 140 hours' incubation the potential remained below $-0{\cdot}15$ v. The potential-time curve obtained may be contrasted with that of an aerobic culture in the same broth of haemolytic streptococci. With the latter organisms the potential falls rapidly to a minimum value of $-0{\cdot}17$ v. and then rapidly rises.

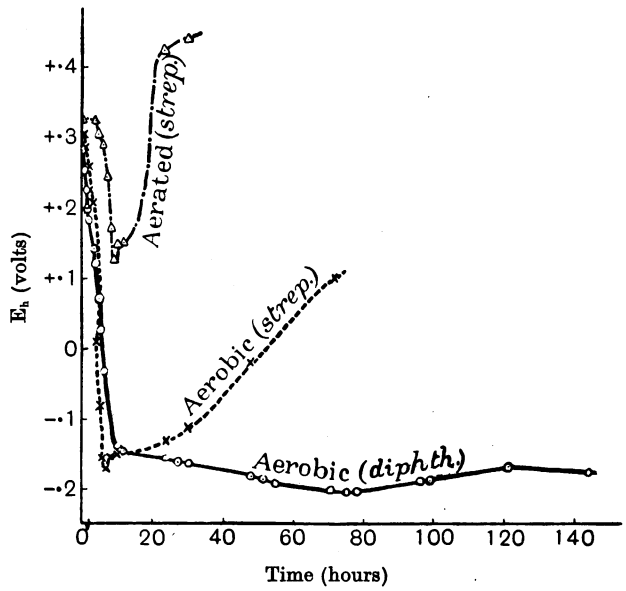

Fig. 1. Peptone infusion broth. *C. diphtheriae* and haemolytic streptococci.

Freshly heated broth (Fig. 2). Peptone infusion broth in tubes was immersed in a boiling water-bath for 30 minutes and cooled immediately before use. In the case of haemolytic streptococci the potentials of cultures in freshly heated broth fell twice as quickly and to a lower level than in untreated broth [Hewitt, 1930]. No appreciable difference, however, was observed between the potential-time curves of cultures of *C. diphtheriae* in heated and unheated broth.

Tryptic digest broth (Fig. 2). *C. diphtheriae* frequently requires acclimatisation to fresh cultural conditions. When, therefore, an inoculum was made from peptone infusion broth cultures to tryptic digest broth, growth was very slow and poor and, in conformity with this, the potential fell extremely slowly. Even after 96 hours' incubation the potential had barely reached the value $E_h = -0{\cdot}05$ v.

Sealed cultures (Fig. 3). In cultures sealed with vaselin to exclude air the potential fell extremely slowly and did not attain the low level reached in

aerobic cultures. This indicates the necessity of the presence of oxygen to enable the organisms to develop intense reducing conditions. In the case of haemolytic streptococci also, access to air was found necessary for the rapid development of intense reducing conditions.

Aerated cultures (Fig. 3). When broth cultures of *C. diphtheriae* were aerated vigorously and continuously in a three-limbed cell by means of Ridley's [1928] mixing apparatus, growth was more luxuriant than in stationary cultures and was distributed throughout the medium and not confined to a surface pellicle. The potential fell to within 50 mv. of the minimum value reached in stationary cultures and then rose fairly slowly towards the level of the original broth. These results are entirely different from those obtained with haemolytic streptococci (indicated in Fig. 1).

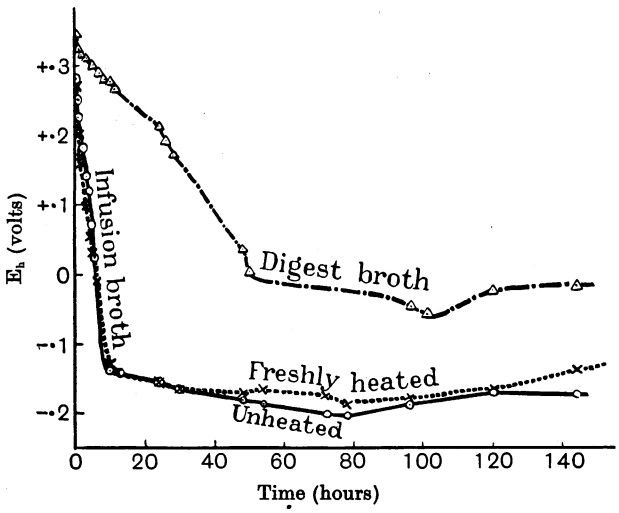

Fig. 2. Aerobic cultures of *C. diphtheriae*.

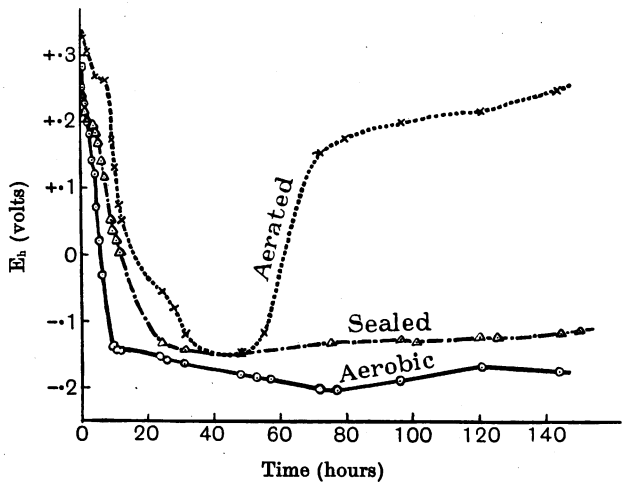

Fig. 3. Peptone infusion broth. Cultures of *C. diphtheriae*.

Table I. *Electrode potential changes in peptone infusion broth.*

Organism	Culture	Maximum fall in potential (mv.)
Streptococci	Stationary	480
,,	Aerated	200
C. diphtheriae	Stationary	500
,,	Aerated	480

With streptococci the fall in potential of aerated cultures was some 40 % of that in stationary aerobic cultures, whilst with *C. diphtheriae* the fall in aerated cultures was fully 90 % of that in stationary aerobic cultures.

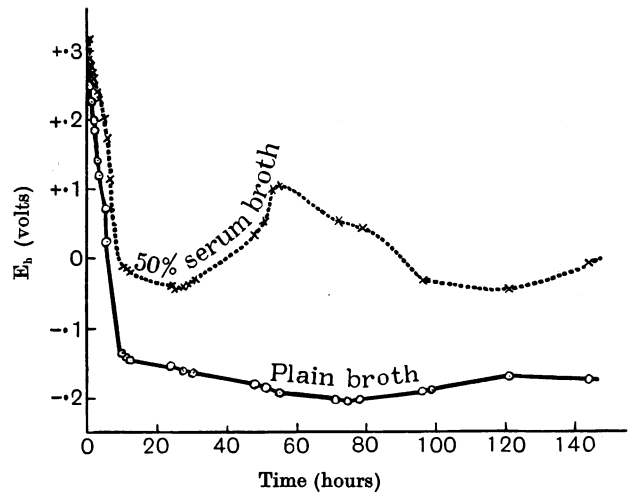

Fig. 4. *C. diphtheriae* cultures.

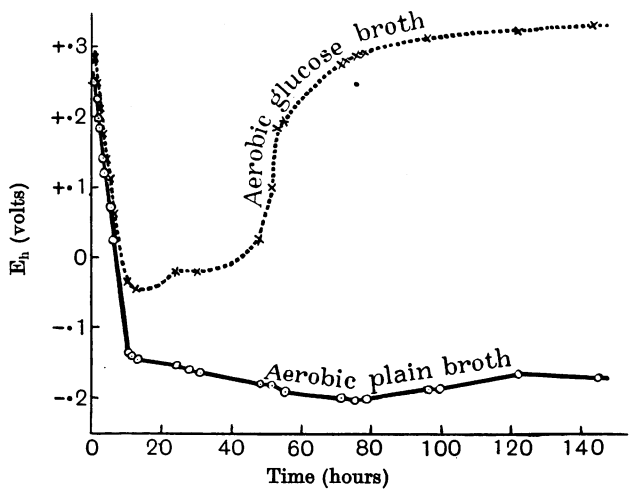

Fig. 5. Infusion broth cultures of *C. diphtheriae.*

After the phase of active growth the potential of streptococcal cultures rises rapidly to a high level, whilst the rise in potential of diphtheria cultures is relatively slow and a high (oxidising) potential is not reached.

Serum broth (Fig. 4). In 50 % serum broth the potential fell much less than in plain broth, as was observed with haemolytic streptococci.

Growth of *C. diphtheriae* appeared to cease much sooner in the presence of serum, but there is some evidence of a second fall in potential after some 60 hours' incubation.

Glucose broth (Fig. 5). For the first two days of incubation active proliferation occurred in 1 % glucose broth, but then ceased owing to accumulation of acid products. The potential did not fall to as low a level as in plain broth. This again is in conformity with results obtained with haemolytic streptococci.

Succinate broth (Fig. 6). Cultures of *C. diphtheriae* in broth containing 1 % of sodium succinate developed a deep yellow-brown colour and became very alkaline owing to oxidation of the succinate to carbonate. The surface pellicle assumed a corrugated shrunken appearance. The level of potential reached was not quite as low as in plain broth cultures and the potential began to rise after 4 days' incubation, possibly owing to the effect of the alkaline reaction on the organisms. In aerated succinate cultures also the brown colour and alkaline reaction were noted, and the potential did not fall to as low a value as in aerated cultures in plain broth.

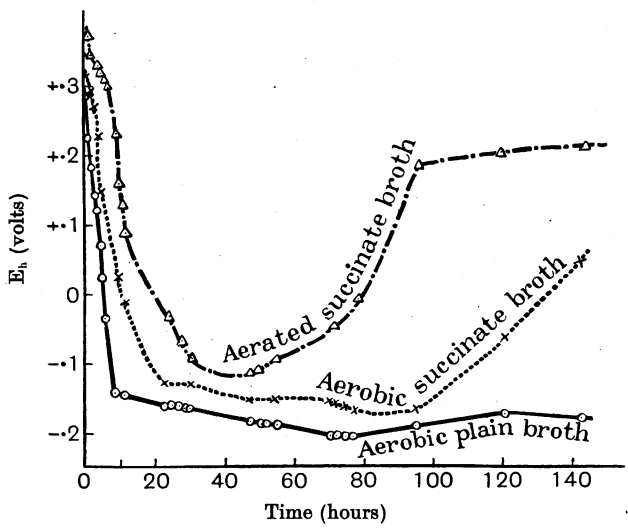

Fig. 6. Infusion broth cultures of *C. diphtheriae*.

Discussion.

In general the potential-time curves of cultures of *C. diphtheriae* are less susceptible to changes in cultural conditions than are those of cultures of haemolytic streptococci. This is probably due to the intense reducing ability of *C. diphtheriae*, which appears to multiply most readily when the oxygen supply is most abundant. As with haemolytic streptococci, the presence of oxygen appears to be necessary for the rapid development of intense reducing

conditions. In cultures sealed with vaselin to prevent access to air the potential falls very slowly. In aerated cultures, in which the oxygen supply is very abundant, growth is luxuriant and the potential falls to very nearly the same low level as in stationary aerobic cultures—the organisms being able easily to deal with the abundant oxygen supply and maintain reducing conditions in the medium until cessation of active proliferation.

The phase of active proliferation of *C. diphtheriae* is longer than that of streptococci and the lowest level of potential is reached more slowly, but the most marked distinction between the behaviour of the organisms is seen after the logarithmic phase of growth. With streptococci after 6–12 hours' incubation the potential begins to rise, but with *C. diphtheriae* no appreciable rise of potential is observed even after 140 hours' incubation. In aerated cultures the high level of potential corresponding to peroxide formation is rapidly attained in streptococcal cultures, but with *C. diphtheriae* this high level is not attained and the potentials do not exceed that of the original uninoculated broth. In the lack of oxidising function *C. diphtheriae* cultures are quite different from those of haemolytic streptococci, and this may be due entirely to the lack of ability to form peroxide.

The presence of serum prevents the potential of cultures of *C. diphtheriae* from falling to the low levels reached in broth. This oxygen-carrying effect, which was attributed tentatively to —S . S— groups in serum-proteins, is therefore not confined to cultures of haemolytic streptococci. Growth in serum broth was not luxuriant with *C. diphtheriae* as with haemolytic streptococci.

The effect of glucose on cultures of *C. diphtheriae* was similar to that on haemolytic streptococci, the acidity produced curtailing growth, but sodium succinate was utilised more readily by *C. diphtheriae* and on oxidation to carbonate produced an alkaline reaction which caused an early rise in potential.

The chief differences between the potentials of cultures of *C. diphtheriae* and haemolytic streptococci have been summarised in the following table. In place of the electrode potential, results are given in terms of the convenient numerical function r_H, which is the logarithm of the reciprocal of the partial pressure of hydrogen which is in equilibrium with the system. Oxidising systems have high r_H and reducing systems low r_H.

Table II. *Approximate values of* r_H *reached in cultures of* C. diphtheriae *and haemolytic streptococci.*

Culture medium	Organism	r_H after 6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	144 hrs.
Unboiled infusion broth ...	*C. diphtheriae*	15	9	8·5	8	7	8
,, ,, ...	Streptococci	18	10·5	14	—	—	—
Freshly heated infusion broth	*C. diphtheriae*	15	9	8·5	8	7	8
,, ,, ,,	Streptococci	9	9	9·5	13·5	18	—
Aerated infusion broth ...	*C. diphtheriae*	22·5	15·5	13	9	21	21
,, ,, ...	Streptococci	20·5	18	30	30	—	—

SUMMARY.

1. More highly reducing conditions are developed in cultures of *C. diphtheriae* than with haemolytic streptococci, especially when the oxygen supply is most abundant.

2. After the logarithmic phase of growth the electrode potentials of cultures of *C. diphtheriae* do not rise rapidly, possibly owing to the lack of a peroxide-forming function.

3. The potential-time curves of cultures of *C. diphtheriae* are less susceptible to variation with cultural conditions than those of haemolytic streptococci, probably owing to the facts stated in the first two paragraphs.

4. Serum possesses an oxygen-carrying effect in cultures of *C. diphtheriae*.

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REFERENCES.

Cannan, Cohen and Clark (1926). *U.S. Pub. Health Reports*, Suppl. **55**.
Coulter and Isaacs (1929). *J. Exp. Med.* **49**, 711.
Fildes (1929). *Brit. J. Exp. Path.* **10**, 151, 197.
Gillespie (1921). *J. Soil Sci.* **9**, 199.
Hewitt (1930). *Biochem. J.* **24**, 512.
Ridley (1928). *Brit. J. Exp. Path.* **9**, 253.