CLXX. OXIDATION-REDUCTION POTENTIALS OF PNEUMOCOCCUS CULTURES. I.

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THE oxidation-reduction potentials established in cultures during the growth of various bacteria under different conditions have been described in previous communications. By extending these studies to other bacteria it is hoped to reveal the general effects of cultural conditions and the individual differences between different organisms.

Serum proteins have an inhibiting effect on the establishment of reducing conditions with each organism studied. The presence of glucose has the same general effect in each case but with individual differences. In aerobic broth cultures haemolytic streptococci [Hewitt, 1930, 1] may be sharply differentiated from *C. diphtheriae* [1930, 2] and staphylococci [1930, 3] by the rise in potential with the first named organisms after the logarithmic phase of growth. With the two latter organisms the potential remains at a low level long after the cessation of active proliferation. A similar difference is observed in aerated broth cultures. With streptococci the potential falls very much less when the cultures are aerated and, after the logarithmic phase of growth, the potential rises rapidly until the high level corresponding to peroxide formation is reached. In the case of diphtheria and staphylococcal cultures aeration has relatively little effect on the level of reduction potential reached, and the potential after the logarithmic phase of multiplication does not rise to a high oxidising level. Since peroxide formation occurs in streptococcal cultures but not in diphtheria or staphylococcal cultures, it is possible that the general character of the potential-time curves may be due to the ability or inability on the part of the organisms to form peroxide. Since the pneumococcus is a peroxide-forming organism it should behave similarly to the streptococcus and differently from *C. diphtheriae* and *Staphylococcus aureus*.

METHODS.

The general methods and apparatus are the same as those previously described [Hewitt, 1930, 1]. 8 cc. of culture medium were used in each case and the inoculum consisted of 0.1 cc. of a 24-hour broth culture of Pneumococcus Type II. The oxidation-reduction potentials developed in the cultures were followed from the time of inoculation throughout the life-cycle of the cultures.

RESULTS.

Peptone infusion broth (Fig. 1). In horse-flesh infusion broth containing 2 % of Difco proteose peptone the potential of pneumococcus cultures fell fairly rapidly, reaching a minimum value of $E_h - 0.16$ v. in 11 hours (in the unheated medium). Immediately after this fall in potential, which occurred during the phase of logarithmic multiplication, the potential began to rise. In the next 24 hours the potential had risen 0.18 v. These results are very similar to those obtained with haemolytic streptococci [1930, 1] but entirely different from those with diphtheria bacilli [1930, 2] and staphylococci [1930, 3].

Fig. 1. *Pneumococcus* Type II. Infusion broth cultures.

Freshly heated broth (Fig. 1). Broth was immersed in a boiling water-bath for 30 minutes and cooled immediately before inoculation, and in this freshly heated broth the potential of cultures fell much more rapidly than in unheated stock medium. This phenomenon was observed with haemolytic streptococci and staphylococci but not with diphtheria.

Aerated cultures (Fig. 1). Vigorous aeration of cultures was effected in three-limbed cells of the type described previously. The cultures were moved up and down the tubes at the rate of twelve cycles a minute. Growth was poor and the potential of aerated cultures showed practically no fall (E_h of the medium at beginning of experiment + 0.349 v.; minimum value reached after 4 hours, + 0.337 v.). After 4–5 hours' incubation the potential increased and reached $E_h + 0.42$ in 10 hours. This level of potential, which corresponds to peroxide formation, is reached by streptococcal cultures but not by staphylococci or diphtheria. The extremely slight fall in potential is characteristic of pneumococci and has not, so far, been observed with other organisms in plain broth cultures.

Tryptic digest broth (Fig. 2). In Douglas's tryptic digest medium the potential did not fall below $E_h - 0.015$ v. as compared with the minimum value of -0.165 v. in infusion broth. This effect, which varies with different samples of digest medium, is seen to a greater or less extent with other organisms, but is very well marked in the case of pneumococci.

Serum broth (Fig. 3). The potential in infusion broth to which an equal

volume of horse blood-serum had been added did not fall to the low level reached in plain broth. This oxygen-carrying effect of serum has been observed with the other organisms studied and appears to be a general phenomenon, although different samples of serum differ in their quantitative effect on the potentials reached.

Fig. 2. *Pneumococcus* cultures.
(Aerobic in unheated broth.)

Fig. 3. Aerobic *Pneumococcus* cultures.

Glucose broth (Fig. 4). Potential-time curves of closely similar general form are obtained in 1 % glucose broth-cultures of each of the four organisms which have been studied. The potential in 1 % glucose broth-culture of pneumococci falls rapidly for some 5 hours, then after a short period of rapid increase in potential, there is a slight fall followed by a gradual rise. The distinctive feature in the behaviour of pneumococci is that the potential falls to practically the same level in 1 % glucose broth as in plain broth whilst with other organisms there is, in general, rather less fall in glucose broth. The discontinuous line in the figure shows the effect of aerating a glucose broth-culture after some 3 hours' incubation. The potential immediately rises fairly rapidly but afterwards shows a gradual fall.

Fig. 4. 1 % glucose-broth *Pneumococcus* cultures.
(Freshly heated medium.)

Fig. 5. *Pneumococcus* cultures
in 1 % lactate broth.

Lactate broth (Fig. 5). In infusion broth-cultures containing 1 % of sodium lactate the potential shows a delayed fall compared with plain broth-cultures but the same low level of potential is reached. Aeration of cultures after

several hours' growth (discontinuous line in Fig. 5) results in fairly rapid increase in potential and the level corresponding to peroxide formation is reached after 24 hours' incubation. This, again, illustrates the rapid production of peroxide by pneumococci.

DISCUSSION.

Pneumococci resemble haemolytic streptococci in several important particulars. The potential of cultures begins to rise directly after the logarithmic phase of proliferation; aeration has a very marked effect on the minimum (*i.e.* most negative) value of potential reached; and the high oxidising level of potential ($E_h + 0.4$ to 0.5) corresponding to peroxide formation is reached in aerated cultures of each organism. In every one of these particulars diphtheria bacilli and staphylococci behave differently. The difference in behaviour of aerated cultures is shown in Fig. 6. Whereas with streptococci and pneumococci the potential in aerated cultures falls relatively little and rises rapidly to

Fig. 6. Aerated broth-cultures.

a high level, with diphtheria and staphylococci the potential falls to a low level and rises relatively little. It seems possible that the property differentiating the two classes of organisms is that of peroxide formation. This suggests that the possession or non-possession of catalase may be a determining factor in the establishment of oxidation-reduction conditions of bacterial cultures. Pneumococci are less readily able to maintain reducing conditions than haemolytic streptococci, which, in turn are far inferior to staphylococci and diphtheria bacilli in the maintenance of reduction effects particularly in aerated cultures.

In general, the effects of cultural conditions on the potentials in pneumococcal cultures are similar to those observed with other organisms. The potential falls more rapidly in cultures in freshly heated broth, as with streptococci and staphylococci, but not with diphtheria bacilli. Tryptic digest broth inhibits to some extent the establishment of reducing conditions. The oxygen-carrying effect of serum also appears to be a general phenomenon. With each organism studied the potential falls less in broth to which blood-serum has

been added, but the quantitative effect seems to vary with different samples of serum. In aerobic glucose-broth cultures the typical curve is obtained, consisting of an initial fall in potential, followed by a sharp temporary rise, a further slight fall and finally a gradual rise. The minimum values of potential reached in 1 % glucose broth-cultures, aerated plain broth-cultures and ordinary aerobic broth-cultures are as follows.

Table I.

Organism	Minimum potentials (E _h) reached in cultures		
	1 % glucose broth	Aerated plain broth	Aerobic plain broth
<i>Pneumococcus</i> II	-0.155 v.	+0.335 v.	-0.160 v.
Haemolytic streptococci	-0.120 „	+0.100 „	-0.170 „
<i>Staphylococcus aureus</i>	-0.085 „	-0.050 „	-0.195 „
<i>C. diphtheriae</i>	-0.045 „	-0.150 „	-0.205 „

It will be noticed that there is little difference between the minimum potentials reached in ordinary aerobic plain broth-cultures, and a much greater difference in aerated plain broth-cultures, but the relative order is the same in each case, diphtheria being the most potent reducer and pneumococcus the least reducing. In 1 % glucose broth however exactly the reverse is the case, the most intense reducing conditions being established in pneumococcal cultures. The question arises whether the opposite effects of the presence of glucose on the one hand, and of aeration on the other, are essential or accidental. Glucose is a reducing substance whilst aeration may be regarded as exposure to a mild oxidising action. It is possible therefore that the differences revealed in Table I may be due to the ease with which staphylococci and diphtheria bacilli maintain reducing conditions when an abundant oxygen supply is available. The presence of oxygen, oxidising substances, etc., is necessary for profuse growth and the establishment of intense reducing conditions in cultures of these organisms. With pneumococci, however, at the other end of the scale, reducing conditions cannot be maintained when the oxygen supply is abundant, and the presence of potential reducing substance such as glucose may assist in the establishment of reducing conditions.

The study of oxidation-reduction potentials in bacterial cultures is beginning to reveal characteristic differences in the behaviour of different organisms and to indicate general phenomena and special effects in the influence of different culture media on the oxidation-reduction conditions of cultures.

It should be mentioned that the effect of p_{H} on the potential-time curves obtained may be neglected. The variations in p_{H} have relatively little effect in comparison with the influence of other factors on the potential. Attempts to correct the potentials for changes in p_{H} are fallacious and give a false impression of accurate correction where none is possible. To correct E_h values for p_{H} variations it is necessary to have an accurate knowledge of the actual oxidation-reduction system studied, and of the dissociation constants of reactants of the system, and of their range of effect. In the case of bacterial cultures none of

these factors is known, and, moreover, it is probable that the direct effect of p_H on the potential is subsidiary to the indirect effect of p_H variations, which will affect the behaviour of the organisms. Repetition and control experiments reveal the insignificance of experimental errors in the phenomena considered. The differences between different organisms and different cultural conditions far outweigh other variations.

SUMMARY.

1. The oxidation-reduction potential-time curves obtained with pneumococcal cultures are generally similar to those obtained with haemolytic streptococci, but differ from those obtained with diphtheria bacilli and staphylococci.
2. Peroxide is formed readily by pneumococci, which are not able easily to maintain intense reducing conditions in the presence of an abundant oxygen supply.
3. The general effects of different culture media, *e.g.* infusion, digest, serum and glucose broth, have been observed with pneumococcal cultures.

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