LXXVII. OXIDATION-REDUCTION POTENTIALS OF STAPHYLOCOCCAL CULTURES. I.

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RESULTS obtained with regard to the oxidation-reduction potentials of cultures of haemolytic streptococci [Hewitt, 1930, 1] and *C. diphtheriae* [Hewitt, 1930, 2] have been reported already. Close similarities in some respects and distinct differences in other points were traced in the behaviour of the two classes of organisms. In this communication the study is extended to *Staphylococcus aureus* cultures with the object of obtaining general rules concerning the oxidation-reduction potential changes occurring in bacterial cultures. Staphylococci, like the organisms previously studied, may be cultivated under a variety of aerobic and anaerobic conditions, but most luxuriant growth is observed when free access to oxygen is provided. The very wide range of values of oxygen tension over which many micro-organisms can multiply contrasts with the relatively narrow limits within which higher organisms can survive. This ease of adaptation to different respiratory conditions confers on bacteria the property of great variation in behaviour.

METHODS.

The methods and apparatus employed have been precisely the same as those previously described [Hewitt, 1930, 1]. 8 cc. of medium were used in each experiment and the inoculum was 0.1 cc. of a 24-hour infusion broth culture of *Staph. aureus*.

RESULTS.

Peptone infusion broth (Fig. 1). In ordinary stock horse-flesh infusion broth containing 2 % of Difco peptone the electrode potential fell to the level, $E_h = -0.14$ v., within 9 hours. After this initial drop, the potential fell slightly during the next 140 hours, showing no tendency to rise. This behaviour is similar to that of cultures of *C. diphtheriae* in which the potential remains at a low level for a long period, but quite different from that of haemolytic streptococci. In the latter case the potential begins to rise immediately after the cessation of active proliferation. A distinct point of inflection on the potential-time curves is seen (Fig. 2) indicating reduction processes occurring in two stages.

Freshly heated broth (Fig. 2). Infusion broth was heated for 30 minutes in a boiling water-bath and cooled immediately before use. The potential fell much more rapidly and to a lower level than in unheated broth. This phenomenon was observed with haemolytic streptococci also, but not with *C. diphtheriae*.

Fig. 1. Aerobic cultures of *Staph. aureus*.

Fig. 2. Aerobic cultures of *Staph. aureus*.

Tryptic digest broth (Fig. 1). The potential fell quite rapidly in cultures in Douglas's tryptic digest medium. With haemolytic streptococci this sample of broth had a slight oxygen-carrying effect but it had very little with staphylococcal cultures.

Aerated cultures (Fig. 3). When cultures were aerated continuously and vigorously by means of Ridley's [1928] mixing apparatus growth was much more luxuriant than in stationary cultures. The potential fell rather more slowly and not to as low a level as in stationary aerobic cultures. The difference in potentials reached in aerated and aerobic cultures is much less than in the case of haemolytic streptococci but greater than with *C. diphtheriae*.

Table I. *Minimum electrode potentials reached in cultures.*

	E_h in volts		
	Streptococci	Staphylococci	<i>C. diphtheriae</i>
Aerobic	- 0.16	- 0.155	- 0.20
Aerated	+ 0.12	- 0.055	- 0.15
Difference	0.28	0.10	0.05

The curve obtained with aerated cultures of haemolytic streptococci is given in Fig. 3 for comparison.

Fig. 3. Broth cultures of *Staph. aureus* and haemolytic streptococci.

Serum broth (Figs. 1 and 2). In 50 % serum broth cultures the potential did not fall to as low a level as in plain broth cultures. The form of the potential-time curves is similar to that obtained with haemolytic streptococci and possesses the same points of inflection. A further interesting observation is the second fall in potential in serum broth cultures after some 48 hours' growth. This may be due to breakdown of serum-proteins by proteolytic enzymes possessed by staphylococci.

Glucose broth (Fig. 4). The potential of aerobic cultures in 1 % glucose broth falls fairly rapidly but not to as low a level as in plain broth, and then rises, probably owing to the accumulation of acid in the culture. This phenomenon was observed also with haemolytic streptococci and *C. diphtheriae*.

In aerated glucose broth cultures the potential did not fall to as low a level as in the stationary glucose broth culture (minimum value $E_h = +0.01$ v. in place of -0.08 v.). After a period of increase in potential, however, probably associated with acid production, the potential fell for a second time and after 3-6 days' growth the potential in the aerated culture was actually lower than in the stationary aerobic culture, despite the oxidising effect of the increased oxygen supply in the aerated culture. It was found that the stationary culture had p_H 5.4, whilst in the aerated culture the p_H was 7.0 and growth was more luxuriant. It is evident, therefore, that the extra oxygen supply in the aerated culture had enabled the organisms to utilise the acids liberated during the glucose fermentation. The oxidation of lactate, etc., to carbonate accounts for the more alkaline reaction in the aerated culture.

Fig. 4. Aerobic cultures of *Staph. aureus*.

Lactate-fumarate broth. In broth containing 1 % of sodium lactate and fumarate the potential fell to the same level as in plain broth but rose fairly rapidly. The reaction did not become alkaline and growth was relatively poor, indicating that the organisms were unable to utilise the acids in stationary aerobic cultures to any great extent.

Glycerol broth (Fig. 5). Good growth was observed in stationary aerobic 1 % glycerol broth cultures and the potential-time curve was practically indistinguishable from that of a plain broth culture. The culture did not become as acid as glucose broth cultures (p_H 6.6 in place of 5.4).

Thiodiglycollate broth (Fig. 5). In broth cultures containing 1 % of sodium thiodiglycollate the potential fell rather more slowly and did not reach the low level reached in plain broth cultures. There were also points of inflection on the curve similar to those obtained with haemolytic streptococci.

DISCUSSION.

Staphylococci multiply most readily under conditions of abundant oxygen supply, and are vigorous reducing agents. In this they resemble *C. diphtheriae*; both organisms are more easily able to maintain intense reducing conditions in the presence of a generous oxygen supply than are haemolytic streptococci. In addition, neither *C. diphtheriae* nor staphylococci form peroxide: therefore the potential-time curves of cultures of these organisms remain flat at a low level of potential after the logarithmic phase of growth, whereas with streptococci the curve immediately begins to rise.

Fig. 5. Aerobic cultures of *Staph. aureus*.

Staphylococci resemble streptococci and differ from *C. diphtheriae* in the effect of freshly heated broth. In the case of the two former organisms the potential falls more rapidly and to a lower level in the freshly boiled broth. With *C. diphtheriae* no effect is seen.

With all three organisms the potential does not fall to as low a level in serum broth cultures as in plain broth. The oxygen-carrying effect of serum-proteins appears therefore to be a general one.

In glucose broth cultures also the three organisms are similar in that the potential does not fall to as low a level as in plain broth. With staphylococci, however, another effect is seen. Acids liberated during glucose fermentation are utilised much more readily in vigorously aerated cultures than in stationary aerobic cultures.

The presence of glycerol appears to have little effect on the potential-time curves of cultures, does not lead to acid production, and is therefore quite different in its effects from that of glucose.

When staphylococcal cultures are sealed autolysis proceeds and the culture clears. Little effect on the potential is observed, which suggests that the potential of the intracellular contents is very little different from that of the medium, under the conditions employed.

For comparison with previous results there are given in the following table the values of r_H (logarithm of reciprocal of partial pressure of hydrogen in equilibrium with the system) reached in *Staphylococcus aureus* cultures.

Nature of culture	Approximate r_H after					
	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	120 hrs.
Unheated broth culture	12	10	10	10	10	9.5
Freshly heated culture	9.7	9.7	9.3	9	8.5	8.0
Aerated broth culture	15	12	12.5	15	20.5	22

SUMMARY.

1. *Staphylococcus aureus* resembles *C. diphtheriae* in maintaining more intense reducing conditions when the oxygen supply is abundant than haemolytic streptococci.

2. Probably owing to lack of peroxide-formation the potential in staphylococcal cultures remains at a low level for a long period, again like diphtheria and unlike streptococci.

3. In freshly heated broth the potential falls more rapidly in staphylococcal cultures, as with streptococci but not with *C. diphtheriae*.

4. Serum has an oxygen-carrying effect in cultures of staphylococci as with the other organisms investigated.

5. It is found with staphylococci, as with streptococci and *C. diphtheriae*, that the potential does not fall to as low a level in glucose broth as in plain broth; but with staphylococci an abundant oxygen supply facilitates the utilisation of acids liberated during glucose fermentation.

6. Glycerol, unlike glucose, has little effect on the potential-time curve.

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