XCVII. OXIDATION-REDUCTION POTENTIALS OF HAEMOLYTIC STREPTOCOCCI.

II. EFFECT OF CATALASE.

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PEROXIDE formation has a marked effect on the electrode potential behaviour of bacterial cultures and it has been shown in previous communications that the course of development of reducing and oxidising conditions in cultures of the peroxide-forming organisms haemolytic streptococci [Hewitt, 1930, 1] and pneumococci [1930, 4] differs characteristically from that in cultures of the catalase-containing bacteria *C. diphtheriae* [1930, 2] and staphylococci [1930, 3]. Certain of the characteristics in the behaviour of pneumococcus disappear when peroxide accumulation is prevented by addition of catalase to the cultures [1931, 1]. Nevertheless differences still remain between the reducing activities of pneumococcus cultures containing catalase on the one hand and, say, staphylococcus cultures on the other.

Hydrogen peroxide is a common by-product in biological oxidation-reductions and has a very definite effect on electrode potentials. It is clear therefore that the presence or absence of catalase (or other peroxide-decomposing system) must have a pronounced effect on the potentials developed in bacterial cultures. It is important, however, to bear in mind that the presence or absence of other enzymes concerned in biological oxidation-reduction reactions must also have marked effects. McLeod [1930] has suggested that the phenomena of anaerobiosis may be ascribed to peroxide formation, but it is probable that other factors are involved [Hewitt, 1931, 2]. It is of interest, therefore, to determine the differences in the oxidation-reduction potentials developed in cultures of different organisms when peroxide formation is eliminated by the addition of catalase, and this short communication is an account of the effect of catalase on the oxidation-reduction potentials of cultures of haemolytic streptococci.

METHODS.

Electrode potentials of cultures were measured by means of the apparatus and methods previously described [1930, 1; 1931, 2]. The glossy variant of Aronson-Schnitzer strain of haemolytic streptococci [Todd, 1930, 1] was used and the inoculum of 0.1 cc. of a 24 hour broth culture was made into 8 cc. of medium in each experiment. Cultures were incubated at 37° and readings of the potential were made every 30 minutes during the first 12 hours' incubation.

The catalase preparation used was prepared from pig's liver by the method of Batelli and Stern [1904]. The liver was extracted with water, the catalase was precipitated by addition of alcohol, filtered off, redissolved in water, reprecipitated by alcohol, filtered off and dried *in vacuo*. Catalase broth was prepared by suspending the highly active catalase preparation in broth in the required concentration, and after standing for an hour the broth was filtered through a sterile Seitz filter. The catalase preparation did not dissolve completely, but the filtered broth decomposed hydrogen peroxide vigorously even after standing at room temperature for 2 months. 1 % and 0.2 % catalase broth were used, that is 1.0 or 0.2 g. of the catalase powder suspended in 100 cc. of broth.

RESULTS.

No differences were detected between the electrode potential of plain broth and that of broth to which the catalase preparation had been added 24 hours previously.

Stationary aerobic cultures (Fig. 1). In plain peptone infusion broth cultures

Fig. 1. Stationary aerobic cultures (peptone-infusion broth).

the potential fell to its minimum value of about -0.1 v. in 12 hours. The potential had risen to $+0.07$ v. in 24 hours and to $+0.16$ v. in 30 hours. This rapid rise in potential after the logarithmic phase of growth is characteristic of peroxide-forming bacteria. In broth containing 0.2 % of the liver catalase preparation the potential fell to a slightly lower level than that of the plain broth culture and after 30 hours' incubation was still below -0.11 v. The catalase had thus inhibited the usual rise in potential after the logarithmic phase of growth and had brought the behaviour of haemolytic streptococci more into line with that of staphylococci or *C. diphtheriae*. The behaviour of pneumococci in the presence of catalase was similar. The disappearance of reducing conditions after the logarithmic phase of growth is evidently associated with peroxide formation, although no peroxide can be detected in aerobic cultures under these conditions.

The presence of high concentrations of the catalase preparation in broth

(e.g. 1 %) produced a definite poisoning effect on the potentials, probably owing to the presence of extraneous substances such as haematin derivatives.

Aerated cultures (Fig. 2). Cultures were aerated in three-limbed cells by

Fig. 2. Aerated digest-broth cultures.

the method previously described [1930, 1]. In the plain tryptic digest broth cultures the potential fell to a minimum level of +0.31 v. in 2.5 hours and then rose rapidly to +0.5 v., at which level peroxide may be detected in the culture. In the presence of 0.2 % catalase however the potential fell to a level of about +0.1 v. and after 30 hours' incubation had not risen above +0.2 v. The addition of catalase, therefore, had prevented peroxide formation, with the result that the potential fell to a much lower level and did not rise to the high level corresponding to peroxide formation. A similar effect was seen in the case of pneumococci [1931, 1].

As a matter of interest a small quantity of the catalase preparation was added to the aerated plain broth culture after 25 hours' incubation. There was evident effervescence of oxygen as the peroxide present was decomposed. Despite this vigorous oxygenation the potential fell rapidly. The fall was 0.1 v. within 10 minutes after the addition of the catalase, and the total fall in 4 hours was 0.2 v. Two conclusions may be drawn from this experiment; first, it is evident that peroxide formation accounted for the high potential since the addition of catalase resulted in an immediate fall; second, the relatively inert nature of molecular oxygen is demonstrated. Although hydrogen peroxide may be regarded as a reduction product of oxygen, yet peroxide has a much higher oxidising potential. The tendency to part with molecular oxygen evidently does not account for the oxidising effect of hydrogen peroxide.

DISCUSSION.

Bacterial peroxide formation is of considerable importance since it has been suggested as a criterion for the classification of bacteria, as an explanation of the behaviour of obligate anaerobes [McLeod, 1930] and as a factor in bacterial variation [Todd, 1930, 1, 2, 3]. The glossy variant of haemolytic streptococci is less sensitive to peroxide than the matt variants, hence there is a natural selection of glossy variants when peroxide is formed. When,

however, peroxide formation is inhibited by the presence of catalase the degradation of matt to glossy variants does not occur [Todd, 1930, 1]. The glossy variants are avirulent, whilst the matt form may be virulent or attenuated, and the virulence is maintained on long continued artificial cultivation only when the oxygen supply is abundant and peroxide accumulation in the culture is avoided [Todd, 1930, 2]. The effect of catalase on the oxidation-reduction conditions developed in cultures of haemolytic streptococci is, therefore, of more than academic interest.

Addition of catalase to the cultures inhibits peroxide formation by haemolytic streptococci and results in corresponding modifications of the electrode potential-time curves. In aerobic catalase broth cultures the potential remains at a low level long after the cessation of active proliferation and does not rise as in plain broth cultures. In aerated cultures the potential falls to a lower level in the presence of catalase and the usual high level corresponding to peroxide formation is not reached. The effect of catalase on cultures of haemolytic streptococci is therefore closely similar to that observed with pneumococci [1931, 1].

When a small quantity of the catalase preparation was added to an aerated culture of haemolytic streptococci in which peroxide had been formed the potential immediately fell very rapidly although oxygen was visibly liberated throughout the culture. This presents confirmatory evidence that

(1) the effect of the enzyme preparation on the potentials of growing cultures was due to catalase activity and not to extraneous factors;

(2) bacterial peroxide is closely similar to, if not identical with, hydrogen peroxide;

(3) peroxide has a much greater effect on the potential than molecular oxygen.

In many respects, cultures of haemolytic streptococci to which catalase has been added behave qualitatively like those organisms which produce their own catalase. Quantitative differences, however, still remain. Thus the potential-time curves of aerated cultures of pneumococci and haemolytic streptococci are entirely different from those of staphylococci and *C. diphtheriae*. When catalase is added to cultures of pneumococci and haemolytic streptococci the potential-time curves are now roughly similar in form to those of the catalase-containing bacteria; but the minimum values of potential reached vary widely, being approximately: pneumococci + 0.15 v., haemolytic streptococci + 0.05 v., staphylococci - 0.05 v. and *C. diphtheriae* - 0.15 v. These figures are only roughly comparable since they were obtained on different occasions with different samples of broth *etc.* They are sufficient, however, to indicate the great differences in reducing activity of these different bacteria. In aerobic cultures not subjected to special aeration the differences are much smaller, the minimum values being approximately pneumococci - 0.16 v., haemolytic streptococci - 0.17 v., staphylococci - 0.18 v. and *C. diphtheriae* - 0.20 v.

Thus, although the formation or non-formation of peroxide may be a valuable criterion for the classification of bacteria and may have important effects on bacterial variation, it should not be regarded as the only determining factor in bacterial behaviour.

SUMMARY.

1. Addition of catalase inhibits peroxide formation in cultures of haemolytic streptococci and the effect is closely similar to that observed with pneumococci.

2. Reducing conditions are maintained after the logarithmic phase of growth when catalase has been added to the culture.

3. The electrode potential falls to a lower level in aerated cultures when catalase is added, and the high level corresponding to peroxide formation is not reached.

4. Addition of catalase to a culture containing peroxide causes an immediate and rapid fall in potential despite the resulting vigorous oxygenation of the culture.

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REFERENCES.

- Batelli and Stern (1904). *Compt. Rend. Soc. Biol.* 57, 374.
Hewitt (1930, 1). *Biochem. J.* 24, 512.
— (1930, 2). *Biochem. J.* 24, 669.
— (1930, 3). *Biochem. J.* 24, 676.
— (1930, 4). *Biochem. J.* 24, 1551.
— (1931, 1). *Biochem. J.* 25, 169.
— (1931, 2). Oxidation-reduction potentials in bacteriology and biochemistry, pp. 46–51. (London.)
McLeod (1930). A system of bacteriology, vol. 1, chap. vii. (Medical Research Council, London.)
Todd (1930, 1). *Brit. J. Exp. Path.* 11, 368.
— (1930, 2). *Brit. J. Exp. Path.* 11, 469.
— (1930, 3). *Brit. J. Exp. Path.* 11, 480.