CLV. EFFECT OF BACTERIOPHAGE ON OXIDATION-REDUCTION POTENTIALS OF *B. DYSENTERIAE* (SHIGA) CULTURES.

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It has been shown in previous communications that the oxidation-reduction potential curves of peroxide-forming bacteria are entirely different from those of catalase-containing organisms [Hewitt, 1930]. Furthermore, when catalase is added to cultures of peroxide-forming bacteria the oxidation-reduction potential curves obtained are similar in many respects to those of organisms which themselves form catalase [1931, 1]. The behaviour of *B. dysenteriae* (Shiga) is of interest in this connection since it neither forms peroxide nor contains catalase [McLeod, 1930]. It is, therefore, of importance to determine in what respects the oxidation-reduction potentials developed in cultures of *B. dysenteriae* differ from those developed in cultures of peroxide-forming bacteria on the one hand and catalase-containing bacteria on the other.

In addition to these points the opportunity occurs with *B. dysenteriae* to study the effect of the bacteriophage phenomenon on oxidation-reduction potentials. It appeared possible that the destruction and lysis of bacteria by the action of bacteriophage might be accompanied by characteristic changes in the oxidation-reduction potentials of the cultures.

With these several objects in view observations have been made of the potentials developed in *B. dysenteriae* (Shiga) cultures and of the effect of bacteriophage.

Methods.

The methods and apparatus used were those described previously [Hewitt, 1930; 1931, 2]. In each experiment 8 cc. of culture medium were inoculated with 0·1 cc. of a 24-hour broth culture of the rough variant of *B. dysenteriae* (Shiga) and the potentials developed were followed during the active growth of the culture. The author is indebted to Dr C. Todd for a sample of Shiga phage.

Results.

Potentials of cultures without phage.

Plain broth cultures (Fig. 1). In stationary aerobic peptone-infusion broth cultures the potential fell slowly for the first 2 hours (lag period) and more

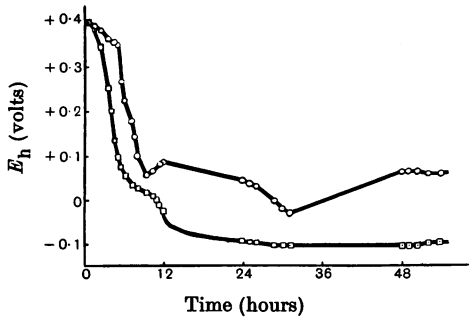

Fig. 1. Infusion broth cultures of *B. dysenteriae* (Shiga).

rapidly for the next 2 hours. The lowest level of potential (− 0·1 v.) was reached after 48 hours' growth and was maintained for several days.

The fall in potential was very sluggish also in cultures which were vigorously aerated by the method previously described [1931, 2]. After a lag period lasting some 4 hours the potential fell to the level + 0·1 v. The fall from this level to + 0·02 v. was very slow as with the stationary aerobic culture. The lowest level of potential (− 0·03 v.) was reached after 30 hours' incubation, and the potential had not risen above the level + 0·06 v. after 54 hours' growth (upper curve, Fig. 1).

B. dysenteriae (Shiga) has, therefore, feeble reducing powers and no evidence was obtained of the high levels of potential corresponding to peroxide formation. The oxidation-reduction potential behaviour resembled that of catalase-containing bacteria except that the reducing powers were less intense.

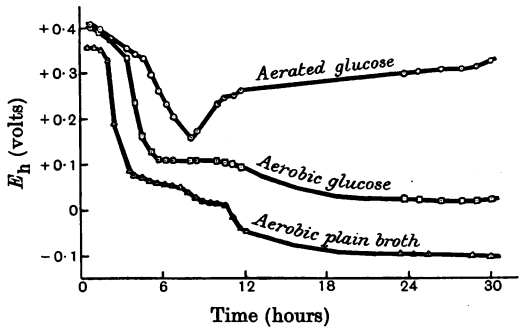

Fig. 2. Cultures of *B. dysenteriae* (Shiga).

1 % glucose broth cultures (Fig. 2). In contradistinction to the behaviour of the other bacteria investigated the potential fell much more slowly in

stationary aerobic glucose broth than in plain broth. The lowest level of potential reached was + 0·02 v. in 25 hours and the potential rose very slowly. With other organisms investigated there has been a rapid fall in potential in glucose broth followed by a rapid rise, then a slight fall and finally a more gradual rise.

In aerated glucose broth cultures the potential reached its lowest level, + 0·16 v., in 8 hours and then rose fairly rapidly. This fall in potential is intermediate between that of haemolytic streptococci (lowest level + 0·2 v.) and that of staphylococci (− 0·01 v.).

Effect of bacteriophage (Figs. 3, 4).

0·1 cc. of Shiga phage was added to an aerobic 7-hour culture of *B. dysenteriae* (Shiga) in infusion broth. After 18 hours' incubation the culture had cleared and was filtered through a small Seitz filter. The bacteriophage was subjected to several passages before it was used.

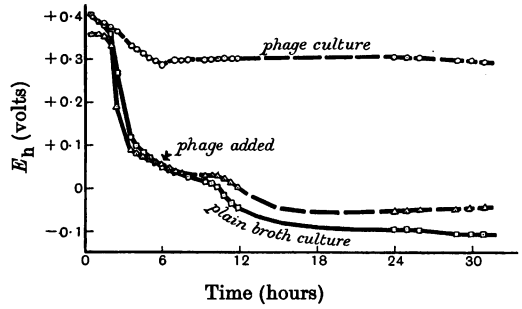

Fig. 3. Stationary aerobic cultures. (Broken lines indicate cultures containing bacteriophage.)

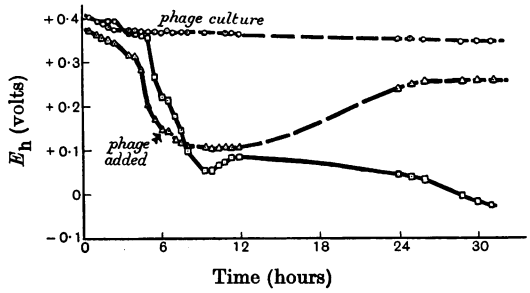

Fig. 4. Aerated broth cultures.

When the phage (0·1 cc.) was added to a culture immediately after inoculation with *B. dysenteriae* the potential fell very little even after 54 hours' incubation, and no visible growth occurred. In neither the stationary aerobic (Fig. 3) nor the aerated cultures did the potential fall below + 0·29 to 0·33 v. It is evident that the dysentery bacilli had not proliferated to any appreciable

extent and that the bacteriophage itself had no detectable effect on the potentials. The bacteriophage had merely inhibited the usual fall in potential accompanying growth of the organisms. In further experiments 0·1 cc. of bacteriophage was added to the culture after 6 hours' incubation. In these experiments the fall in potential slackened after the bacteriophage had been added. After a further 6 hours' incubation in aerated cultures (Fig. 4) and after 18 hours in stationary aerobic cultures the potential had commenced to rise. The bacteriophage appeared to have no effect other than that of inhibiting the proliferation and hence the metabolic changes and fall in potential of the cultures.

Discussion.

B. dysenteriae (Shiga) possesses only feeble reducing powers compared with other bacteria investigated. The potentials of cultures of this organism fall fairly slowly and do not reach the low levels attained by haemolytic streptococci, *C. diphtheriae*, staphylococci and pneumococci [Hewitt, 1930]. Although the Shiga bacillus does not contain catalase, it does not display the electrode potential behaviour of peroxide-forming organisms, but behaves similarly to *C. diphtheriae* and staphylococci, which contain catalase. This general similarity to the catalase-containing organisms is shown in Fig. 5.

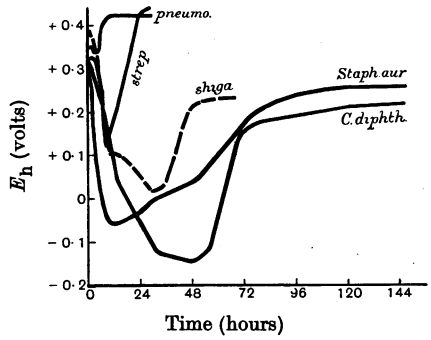

Fig. 5. Aerated broth cultures of different bacteria.

Hydrogen peroxide is formed in many biological oxidation-reduction reactions and cells of many kinds contain catalase and, hence, are able to destroy the toxic peroxide as it is formed. *C. diphtheriae* and staphylococci are examples of this type. Haemolytic streptococci and pneumococci, on the other hand, do not contain catalase and hence peroxide accumulates in aerated cultures of these bacteria. Addition of catalase to their cultures inhibits peroxide formation and certain of the characteristic features of their electrode potential behaviour disappear [Hewitt, 1931, 1]. It is remarkable therefore that the Shiga bacillus, which does not contain catalase, should not behave like a peroxide-forming organism. It is possible that the explanation of the anomaly lies in the fact that *B. dysenteriae* (Shiga) displays peroxidase activity, possibly associated with its cytochrome content [Keilin, 1929] and

thus any peroxide formed in the culture may react, in the presence of peroxidase, with broth constituents, and hence be removed as it is formed.

This is of interest from the point of view of the classification of bacteria. McLeod [1930] has classified bacteria according to their possession or non-possession of catalase and their degree of sensitivity to peroxide. As has been pointed out elsewhere [Hewitt, 1931, 2] other enzymes than catalase must affect the proliferation of bacteria under different conditions of oxygen supply, and it now seems possible that peroxidase activity may be of considerable importance in bacteria which do not contain catalase, as it may provide an alternative method of removing the toxic effects of peroxide accumulation.

Todd [1930] found that in the case of haemolytic streptococci peroxide still accumulated but in smaller amounts when peroxidase was added to the cultures. The question remains unsettled, therefore, as to whether it is the presence of peroxidase or some other difference in respiratory mechanism which accounts for the absence of peroxide formation by the Shiga bacillus.

Bacteriophage itself appears to have no effect on the oxidation-reduction potential of culture media. When added to the cultures at the time of inoculation Shiga phage inhibits the usual fall in potential accompanying the growth and metabolic activities of *B. dysenteriae* (Shiga). Thus the inhibition of growth is reflected in the lack of fall in potential. When the bacteriophage is added after the culture has grown the potential gradually ceases to fall and commences to rise as the bacteria and their metabolic activities are destroyed.

Summary.

1. *B. dysenteriae* (Shiga) possesses relatively feeble reducing powers.

2. Although it does not contain catalase its behaviour resembles that of catalase-containing organisms and not that of the peroxide-forming bacteria.

3. This behaviour may be due to peroxidase activity which is probably of some importance in the classification of bacteria.

4. Bacteriophage itself appears to have no effect on the oxidation-reduction potential but it inhibits the usual fall in potential of *B. dysenteriae* cultures by inhibiting the growth and metabolic activities of the bacteria.

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