CLXXVI. EFFECT OF BACTERIOPHAGE ON THE OXIDATION-REDUCTION POTENTIALS OF *B. COLI COMMUNIS* CULTURES.

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(*Received August 15th, 1931.*)

Addition of bacteriophage to cultures of *B. dysenteriae* (Shiga) has the effect of inhibiting the fall in potential associated with the growth and metabolic activities of the organisms [Hewitt, 1931, 1]. The oxidation-reduction potentials developed in cultures of *B. coli communis* and the effect thereon of bacteriophage are described in this communication.

Methods.

The general methods and apparatus used have been described previously [Hewitt, 1931, 2]. In each experiment 8 cc. of culture medium were inoculated with 0·1 cc. of a 24-hour broth culture of *B. coli*. Dr C. H. Andrewes kindly provided a sample of bacteriophage and a culture of *B. coli communis* which was sensitive to the action of the bacteriophage. Before use the bacteriophage was subjected to several passages by adding it to 6-hour broth cultures of *B. coli* which were incubated for a further 18 hours and then filtered through small Seitz filters.

Results.

Cultures without bacteriophage.

Plain broth cultures. In peptone-infusion broth the potential of stationary aerobic cultures of *B. coli* fell rapidly, reaching its lowest level, E_h − 0·28 v., in 3½ hours. The low level of potential reached is probably due to liberation of hydrogen, visible liberation of gas in the cultures being observed after about 4 hours' incubation.

In vigorously aerated broth cultures there was a slightly longer lag period before the potential began to fall rapidly, but the lowest level of potential was reached in 3½ hours as with the stationary aerobic culture. The lowest level of potential noted in the cultures examined (− 0·36 v.) was somewhat lower than in stationary aerobic cultures. In cultures at a low potential, in which hydrogen liberation would be expected, the potential was observed to vary during the pulse of the aeration apparatus. The aeration apparatus was working at the rate of 10 cycles per minute and the potential rose during each

aeration stroke and then gradually fell again until the next stroke. The fluctuation varied from a few millivolts to nearly 0·1 v. Presumably hydrogen was being liberated rapidly and during each aeration stroke a portion of the hydrogen in the culture was displaced by air. The fact that as low a potential as − 0·36 v. could be established in a vigorously aerated culture illustrates the intense metabolic and enzymic activity displayed by *B. coli communis*.

Since *B. coli* contains catalase it was not surprising to find that no evidence could be obtained of the establishment of the high potentials corresponding to peroxide formation.

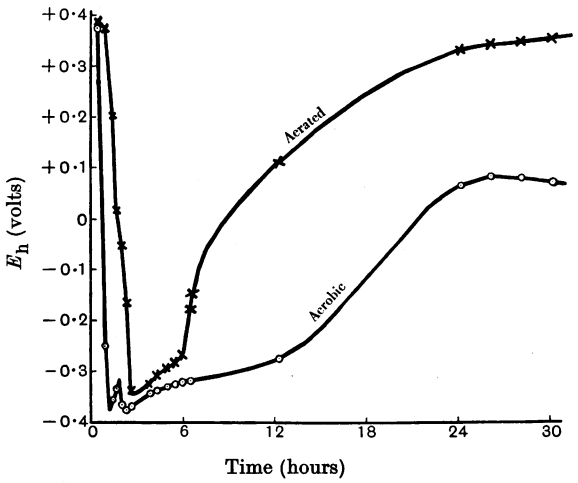

Fig. 1. Glucose broth cultures of *B. coli*.

Glucose broth cultures (Fig. 1). The fall in potential in 1 % glucose broth cultures was the most rapid yet recorded. In stationary aerobic cultures the potential had reached its lowest level (− 0·38 v.) in 2 hours. The most rapid fall in potential observed was 0·65 v. in 30 minutes. The kink in the potential-time curve which occurred in this case after 1 hour's incubation has been observed also with haemolytic streptococci.

In aerated glucose broth cultures the fall in potential was only slightly less rapid than in stationary aerobic glucose broth cultures, but after the logarithmic phase of growth the potential rose very rapidly.

Effect of bacteriophage on potentials.

0·1 cc. or 0·25 cc. of bacteriophage was added to each cell containing 8 cc. of culture.

Plain broth cultures. In stationary aerobic broth cultures containing 0·1 cc. of bacteriophage (Fig. 2) the potential fell to the level + 0·05 v. where it was maintained for some hours. The potential then began to fall again and

after 24 hours' incubation the low level reached in cultures without bacteriophage had been attained.

In aerated broth cultures containing 0·1 cc. of bacteriophage (Fig. 3) there was a delay of 6 or 7 hours before the potential fell below the level + 0·2 v. but after 12 hours' incubation the potential had reached almost as low a level as in aerated cultures not containing bacteriophage.

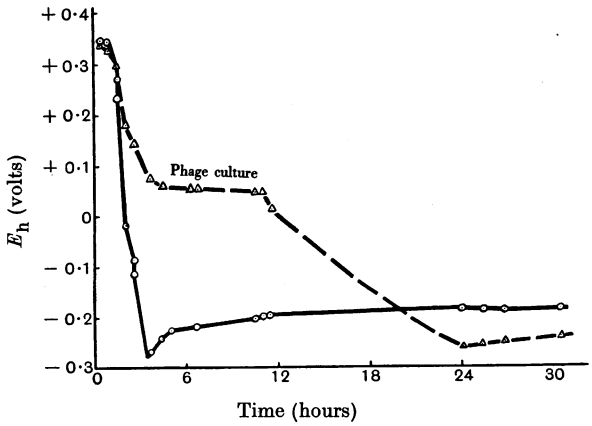

Fig. 2. Aerobic broth cultures.

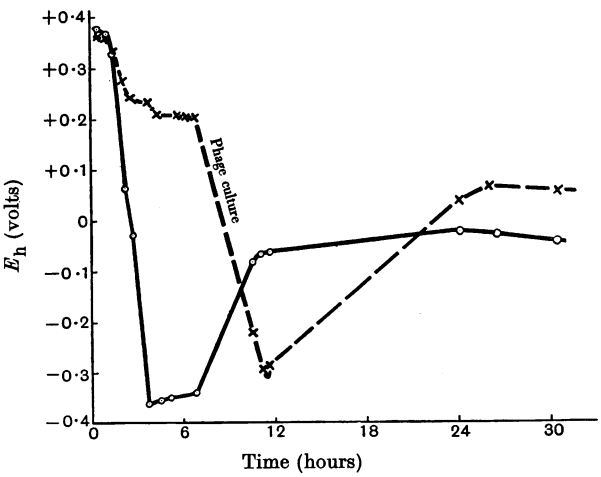

Fig. 3. Aerated broth cultures.

Glucose broth cultures. In stationary aerobic 1 % glucose broth cultures containing 0·25 cc. of bacteriophage (Fig. 4) the initial fall in potential was as rapid as in cultures without bacteriophage, but after this initial fall to a low level there was a rapid rise of potential. Later there was a further fall in potential followed by another rise and fall. Both growth and gas formation are visible in 6-hour glucose broth cultures to which bacteriophage has not

been added, but in cultures containing bacteriophage neither growth nor gas formation is observed until much later (see p. 1646).

In aerated glucose broth cultures containing bacteriophage (Fig. 5) the initial fall in potential was comparatively slight and not as rapid as in cultures without bacteriophage. The initial fall in potential was followed by a rise

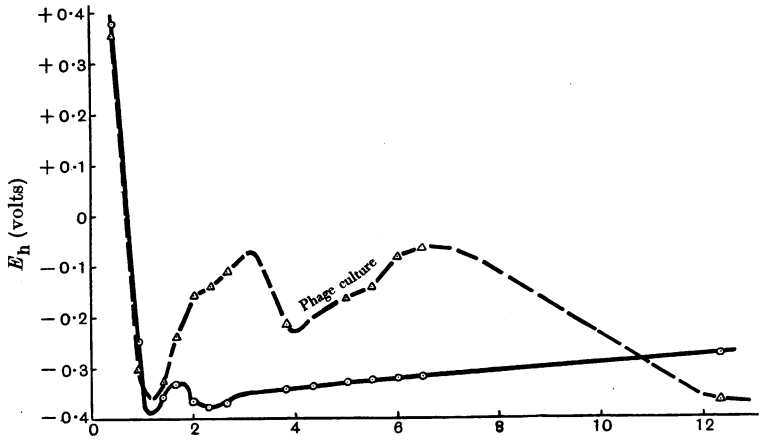

Fig. 4. Aerobic glucose broth cultures.

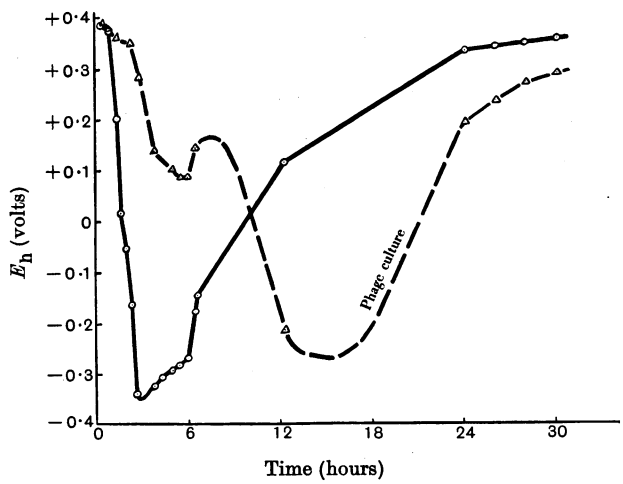

Fig. 5. Aerated glucose broth cultures.

and a subsequent fall to a low level when growth in the culture became visible. The reducing activity of the non-proliferating organisms was not sufficient to overcome the oxidising effect of the extra oxygen supplied to the culture by means of the aeration apparatus, but subsequently, when the organisms had proliferated sufficiently to produce visible growth in the culture, the usual fall in potential occurred.

Discussion.

The electrode potential in cultures of *B. coli* attains lower levels than have been described with other bacteria in this series of communications. These low potentials are associated with the mechanism possessed by *B. coli* for the liberation of hydrogen in its cultures. It may be noted that the potential produced at an electrode in the presence of one atmosphere pressure of hydrogen at p_H 7·0 is − 0·43 v. (at 37°) and in the present series of observations a potential of − 0·36 v. was observed in a culture subjected to aeration. In addition to the attainment of low levels of potential the rate of fall of potential is very rapid in *B. coli* cultures. In 1 % glucose broth cultures the fall is particularly rapid and the minimum level of potential (*ca.* − 0·37 v.) is reached in just over an hour.

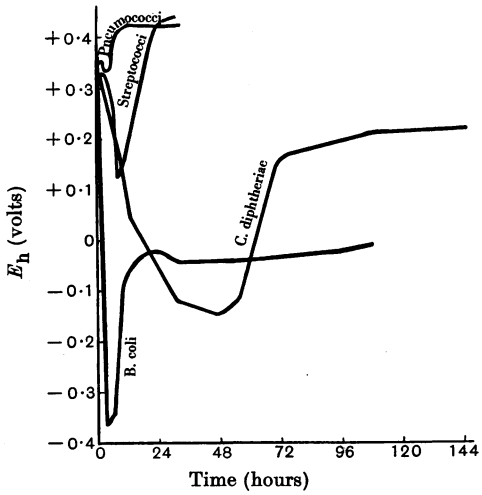

Fig. 6. Aerated broth cultures.

The characteristic behaviour of *B. coli* can be seen in Fig. 6 which gives the electrode potential time curves of aerated broth cultures of three classes of bacteria. Peroxide-forming organisms are represented by a pneumococcus and haemolytic streptococcus, catalase-containing organisms by *C. diphtheriae* and gas-forming organisms by *B. coli*.

The appropriate bacteriophage almost completely inhibits the fall in potential of *B. dysenteriae* (Shiga) cultures [Hewitt, 1931, 1], but in the case of *B. coli* the fall in potential is merely delayed and the potential eventually reaches approximately the same low level in the presence or absence of bacteriophage. The same observation was made with regard to the effect on the growth of the cultures. In the presence of the samples of bacteriophage used in these experiments no growth of *B. dysenteriae* (Shiga) was observed, but with *B. coli* visible growth was merely delayed when bacteriophage had been added. The results reported support the view previously expressed in

the case of *B. dysenteriae* [Hewitt, 1931, 1] that bacteriophage appears to have no effect on the oxidation-reduction potential of cultures except that it inhibits the usual fall in potential accompanying the proliferation and metabolic activities of the bacteria.

An interesting phenomenon was observed in stationary aerobic glucose broth cultures to which bacteriophage had been added. Despite the presence of bacteriophage the potential fell initially as rapidly as in its absence although the growth of the organisms was delayed. After this initial fall the potential rose fairly rapidly but fell again when active proliferation of the bacteria occurred. It is evident that the enzymes present in the bacteria inoculated into the medium were able to activate the glucose present and to establish temporarily intense reducing conditions. The re-establishment of low levels of potential occurred only when the numbers of bacteria present had increased greatly. In aerated glucose broth cultures containing bacteriophage the activity of the bacterial enzymes was insufficient to produce a large initial fall in potential and intense reducing conditions were not established until visible growth had occurred in the culture.

The general effects of the appropriate bacteriophages on the potentials of cultures of *B. dysenteriae* (Shiga) and *B. coli* are similar except that in the case of the Shiga bacillus the bacteriophage completely inhibits growth and the fall in potential, whilst in the case of *B. coli* proliferation and the fall in potential are merely delayed.

Summary.

1. The potential of *B. coli* cultures falls to a lower level and much more rapidly, especially in the presence of glucose, than in the case of other bacteria investigated. This is attributed to liberation of hydrogen in the culture and to a generally intense enzyme activity.

2. In the presence of bacteriophage the fall in potential and also the proliferation of the bacteria are delayed but not completely inhibited as in the case of *B. dysenteriae* (Shiga).

3. The effect of bacteriophage on the oxidation-reduction potential follows the effect on the proliferation of the bacteria, except that in aerobic glucose broth cultures of *B. coli* containing bacteriophage there is an initial fall in potential without appreciable bacterial growth, possibly due to activation of glucose by the enzymes of non-proliferating bacteria.

The author is indebted to Dr C. H. Andrewes for the culture and bacteriophage used and to Dr R. G. White and Dr E. W. Todd for their help and interest.

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Hewitt (1931, 1). *Biochem. J.* **25**, 1447.

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