CCXXIV. OXIDATION-REDUCTION POTENTIALS OF *STAPHYLOCOCCUS* CULTURES.

II. EFFECT OF BACTERIOPHAGE.

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IN the presence of the appropriate bacteriophage the fall in potential of *B. dysenteriae* (Shiga) cultures is completely inhibited whilst in the case of *B. coli* [Hewitt, 1931, 1] the fall in potential is merely delayed. These effects on the oxidation-reduction potential follow the effect on the proliferation of the bacteria, since growth is almost completely inhibited by bacteriophage in the case of *B. dysenteriae* (Shiga) but is merely delayed in the case of *B. coli*. It was concluded therefore that the effect of bacteriophage is merely to inhibit or delay the fall in potential associated with the growth and metabolic activities of the bacteria.

The oxidation-reduction potentials observed in *Staphylococcus aureus* cultures have already been described [Hewitt, 1930] and this paper describes the effect of bacteriophage on the potentials of the cultures.

GENERAL METHODS.

The general methods and apparatus used were those described previously [Hewitt, 1931, 2]: 0.1 cc. of a 24-hour broth culture of *S. aureus* was inoculated into 8 cc. of medium, and the potentials were measured periodically in each experiment.

The sample of bacteriophage and sensitive strain of *S. aureus* used were kindly provided by Dr C. H. Andrewes. Before use the bacteriophage was "subcultured" several times by adding it to 6-hour broth cultures of *S. aureus*, incubating for a further 18 hours and then filtering through Seitz filters. In each experiment 0.25 cc. of phage filtrate was added to each 8 cc. of culture.

RESULTS.

The oxidation-reduction potential curves of the cultures without bacteriophage were substantially the same as those described previously [Hewitt, 1930] with a different strain of *S. aureus*. The effect is described below of the addition of bacteriophage to the cultures both at the time of inoculation with the organisms and after 5 hours' incubation.

Stationary aerobic cultures (Fig. 1). The potential during the first 6 hours' incubation fell as rapidly in the presence of bacteriophage as in its absence, but after this, whereas the potential in the culture without bacteriophage continued to fall and reached the level $E_h - 0.18$ v., in the phage culture the potential rose slightly and did not at any time fall below the level -0.10 v. It will be seen later that these effects, similar in kind but exaggerated in degree, are observed also in the case of aerated cultures. When bacteriophage

Fig. 1. Stationary aerobic broth cultures of *Staphylococcus aureus*. I. Phage added at beginning of incubation. II. Phage added after 5 hours' incubation. III. No phage added.

was added to a *Staphylococcus* culture after 5 hours' incubation the potential of the culture continued to fall for a time but then ceased to fall, and the lowest level of potential reached (-0.12 v.) was intermediate between that of the culture without bacteriophage and that of the culture to which bacteriophage had been added at the beginning of incubation. At the end of the first 12 hours' incubation marked growth was visible in the culture not containing bacteriophage; no growth was visible in the culture to which bacteriophage had been added at the beginning of incubation, and there was very faint growth in the culture to which bacteriophage had been added after 6 hours' incubation. In the culture to which bacteriophage had been added at the beginning of incubation a faint trace of growth was observed first after 30 hours' incubation and it will be seen in Fig. 1 that after this period a slight fall in potential occurred. The initial fall in potential had occurred without marked proliferation of the organisms. A similar phenomenon was observed in the case of *B. coli* but not with *B. dysenteriae* (Shiga) [Hewitt, 1931, 1]. It was observed that cultures to which bacteriophage had been added were more acid than those not containing bacteriophage after 6 days' incubation. This will be commented upon later.

Aerated cultures (Fig. 2). It was found in aerated cultures, as in stationary aerobic cultures, that the presence of bacteriophage had no appreciable effect on the potential during the first 5 hours' incubation, but after this period the potential began to rise rapidly in the cultures containing bacteriophage whilst in the cultures without bacteriophage the potential continued to fall slowly. After 24 hours' incubation the potential in the phage culture had fallen again and at this period growth was first visible. In the culture not containing

bacteriophage growth was observed after only 6 hours' incubation. In the culture to which bacteriophage was added after 5 hours' incubation the potential continued to fall for a time but then rose rapidly and later fell again. This second fall in potential was accompanied by proliferation of the bacteria. The hump-shaped potential-time curves appear to be characteristic of the aerated phage cultures, and are associated with changes in the proliferation of the organisms.

Fig. 2. Aerated broth cultures of *S. aureus*. I. Phage added at beginning of incubation. II. Phage added after 5 hours' incubation. III. No phage added.

DISCUSSION.

The effects of bacteriophage on the oxidation-reduction potentials of *S. aureus* are entirely different from the effects observed with *B. dysenteriae* (Shiga) but are similar in some respects to those observed with *B. coli* [Hewitt, 1931, 1]. In the case of the Shiga bacillus proliferation of the organisms and the associated fall in potential are almost completely inhibited in the presence of bacteriophage, but in the case of *B. coli* proliferation and the fall in potential are merely delayed. On the whole the effect of bacteriophage on the potentials of cultures follows the effect on the growth of the organisms. An exception to this correlation between growth and the fall in potential occurs in the case of stationary aerobic 1 % glucose broth cultures of *B. coli* containing bacteriophage in which the potential shows an initial fall despite absence of bacterial growth. In the case of *S. aureus* also there is an initial fall in potential of cultures in the presence of bacteriophage without appreciable proliferation of the bacteria both in aerobic and aerated plain broth. In aerated cultures containing bacteriophage the potential rises rapidly after this initial fall but later it falls again when visible growth becomes apparent in the culture. The initial fall in potential appears to be due to the enzymic activities of non-proliferating bacteria, but the subsequent fall occurs only when marked growth of the culture takes place. This succession of alterations in potential, namely a fall, rise and fall, gives the potential-time curves of aerated cultures a typical hump-shaped appearance. In stationary aerobic cultures the potential-time curves follow a similar course to those of aerated cultures

except that the rise in potential after the initial fall is much less marked since the oxygen supply is more restricted.

There is a fall in potential during the lysis of *M. lysodeikticus* by lysozyme [Hewitt, 1931, 1] and it was tentatively suggested that this fall in potential might be due to the activity of the enzymes of the lysing bacteria. The fact that in cultures of *S. aureus* containing bacteriophage an initial fall in potential is observed without visible growth of the bacteria suggests that the enzymes of non-proliferating bacteria may be producing this fall in potential and lends some support to the hypothesis that the fall in potential during lysis of *M. lysodeikticus* is a manifestation of the enzymic activity of the lysing bacteria.

After several days' incubation *Staphylococcus* cultures containing bacteriophage are frequently more acid than the cultures not containing bacteriophage. This observation may be accounted for by the fact that during the initial phases of growth bacterial cultures in general become acid owing to production of fatty acids *etc.*, but in later stages of growth of some organisms the reaction becomes more alkaline owing to the oxidation of the acids to carbonates. The more acid reaction of many cultures containing bacteriophage may be due to normal occurrence of the acid phase of metabolism and inhibition by the bacteriophage of the subsequent alkaline phase. This hypothesis is supported by the observation that in *S. aureus* cultures the initial fall in potential occurs in the presence of bacteriophage but the subsequent course of the potential-time curve is altered when bacteriophage is present.

From experiments on *B. coli* Young [1929] concludes that oxidations may be effected by organisms which are dead as judged by their reproductive power.

SUMMARY.

1. The potential of *S. aureus* cultures falls initially even in the presence of bacteriophage despite the delay in the proliferation of the bacteria.
2. In cultures containing bacteriophage the potential soon ceases to fall and commences to rise, but a further fall in potential occurs at the time when growth first becomes apparent in the cultures.
3. Apparently the second fall in potential, but not the initial fall, is dependent on the proliferation of the bacteria.

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