THE RELATIONSHIP BETWEEN BACTERIAL GROWTH AND PHAGE PRODUCTION

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Earlier work on the bacterium-bacteriophage reaction (1) has stressed the importance of bacterial growth as a conditioning factor for phage production. It was found that the rate of phage formation could be expressed in terms of the rate of bacterial reproduction and from this single differential equation there were derived integral forms predicting satisfactorily the time of lysis, number of bacteria present when lysis begins, etc. In the basic equation the rate of phage production was expressed as a power of the rate of bacterial reproduction; *i.e.*, in a mixture of phage and growing bacteria the ratio of phage to bacteria continually increases. Finally, when a certain threshold value is attained (100–140 activity units per bacterium) the process of cellular dissolution or lysis begins. Other workers have since reported practically identical kinetic mechanisms for other phages and other bacteria (2–3).

Despite the fact that the available experimental evidence indicated bacterial growth to be the prime conditioning factor for phage production there remained the possibility that under some conditions the two phenomena might be dissociated. This possibility was confirmed by Scribner and Krueger (4) in an investigation of the reaction between phage and susceptible bacteria in the presence of 0.25 molar NaCl. Under these special conditions a prolonged maximal stationary phase of bacterial growth occurred and during this time phage continued to be produced at the usual rate.

We have conducted further experiments dealing with the effect of temperature and pH on the phage-bacterium reaction, and have

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found that bacterial growth definitely is not the essential conditioning factor for phage formation.

Methods

1. The medium used throughout these experiments was beef infusion broth containing 1 per cent Difco peptone, 0.5 per cent NaCl and was adjusted so that the final pH after sterilization was 7.4.

2. The bacteriophage and bacteria were the same ones used in previous studies.[1] The phage contained 1×10^{10} activity units/ml. The staphylococcal suspensions for each experiment were prepared from eighteen (18) hour cultures grown on nutrient agar in Roux flasks and harvested in broth.

3. The quantitative determination of bacteriophage was carried out by the activity method of Krueger (5). The accuracy of the method, which has been used in this laboratory for the past 6 years, is $\pm$ 5 per cent.

4. In studying the effects of different temperatures on the phage-bacterium reaction a mixture containing 2×10^7 bacteria/ml. and 1×10^7 phage units/ml. in 100 ml. of broth at pH 7.4 was placed in the water bath shaker at the desired temperature. 5.0 ml. samples were withdrawn every 0.3 hour for determining [bacteria] by the turbidity comparison method described in an earlier paper (5). At intervals of 0.4 hour 0.5 ml. of the mixture was pipetted into 4.5 ml. of iced broth. These 1/10 dilutions were kept in ice water until the end of the experiment and were then used for determining [phage].

Controls consisted of phage solutions containing known concentrations of active phage which were exposed to the temperatures used for 3 hour periods. No inactivation of phage was detected in any of the solutions.

5. To determine the effect of various H-ion concentrations on the phage-bacterium reaction normal NaOH or normal HCl was added to the broth in sufficient quantity to produce the desired change in pH. Mixtures of phage and bacteria in 100 ml. amounts of the broth were made so that the initial bacterial concentration was 2×10^7 cells/ml. and the initial phage concentration was 1×10^7 activity units/ml. pH was determined by the glass electrode method at the beginning and at the end of each experimental period. It was found that the broth normally exerted sufficient buffer action to maintain constant pH and no additional buffer mixtures were used. The phage-bacteria mixtures were shaken in the water bath at 36°C. and samples were taken at intervals for determination of [bacteria] and [phage] as described under paragraph 4 above. As controls, to determine the effect of each pH value on phage at 36°C., solutions containing known amounts of phage were adjusted to various H-ion concentrations and were shaken in the water bath for 3 hour periods. It was found that there was no detectable inactivation of bacteriophage under these conditions.

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[1] Reported for the most part in the *Journal of General Physiology* and the *Proceedings of the Society for Experimental Biology and Medicine* from 1929 on.

RESULTS

1. The Effect of Temperature on the Phage-Bacterium Reaction

Figs. 1, 2, 3, and 4 show the course of bacterial growth and phage production at 30°C., 35°C., 40°C., and 45°C. respectively. At 30°C.

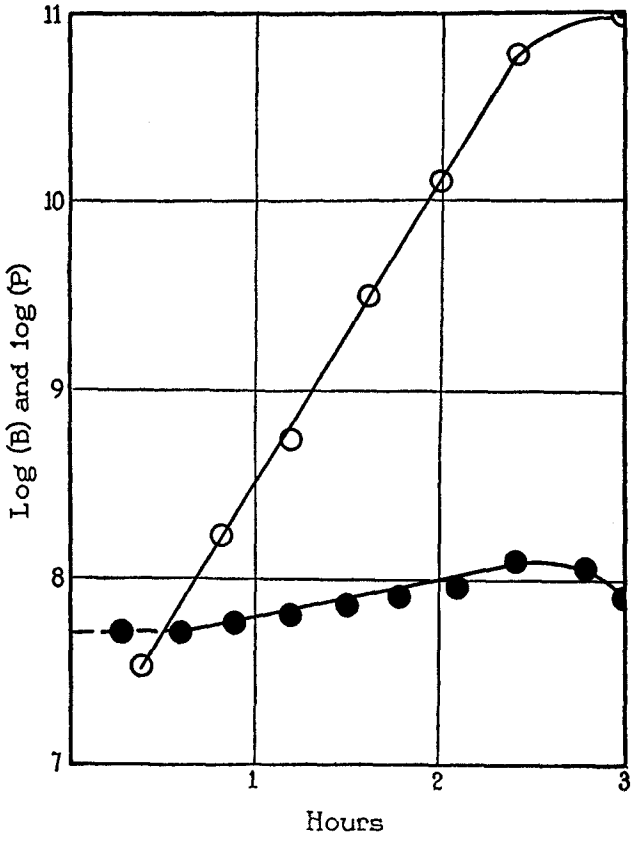

FIG. 1

FIGS. 1–4. The effect of temperature on the phage-bacterium reaction. Logarithms of [bacteria] and [phage] are plotted against time. ○ = phage activity units/ml. ● = bacteria/ml. FIG. 1. Temperature 30°C.

the bacterial population increases 1.5-fold each hour; during the same interval phage concentration increases 40-fold. With a 5° temperature increment the bacterial population shows an increase of 2.5-fold

per hour and the phage concentration a corresponding increase of 160-fold. That is, while the rate of bacterial reproduction rises

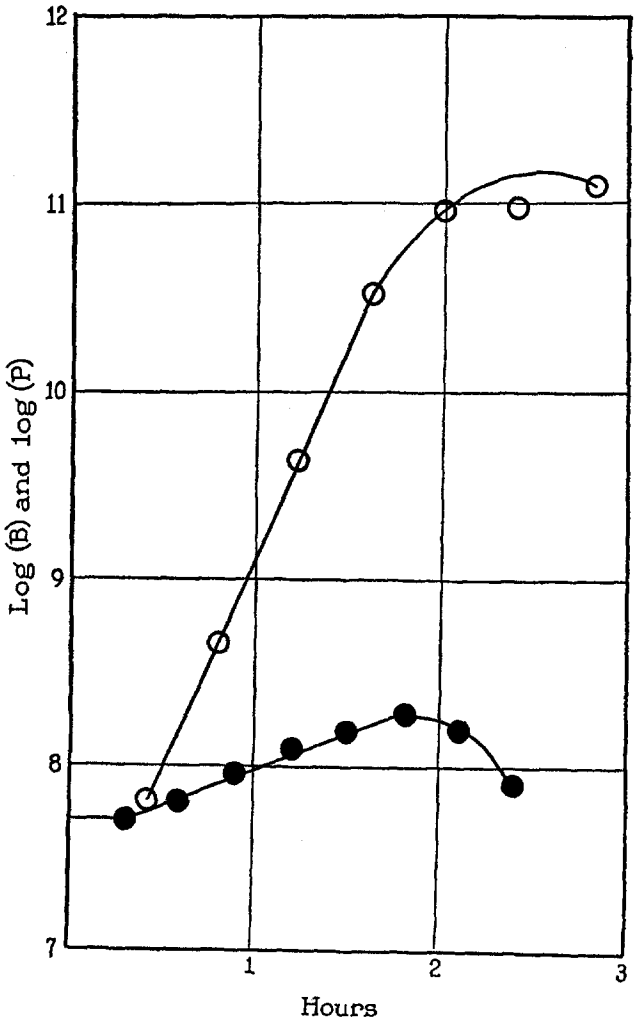

FIG. 2. Temperature 35°C.

about 1.7 times, the rate of phage production increases 4 times. It is interesting that a further rise in temperature to 40°C. brings the rate of bacterial reproduction up to a 3.5-fold increase per hour or about 2.3

times the rate at 30°C. whereas the rate at which phage concentration increases drops to a 25-fold increase per hour.

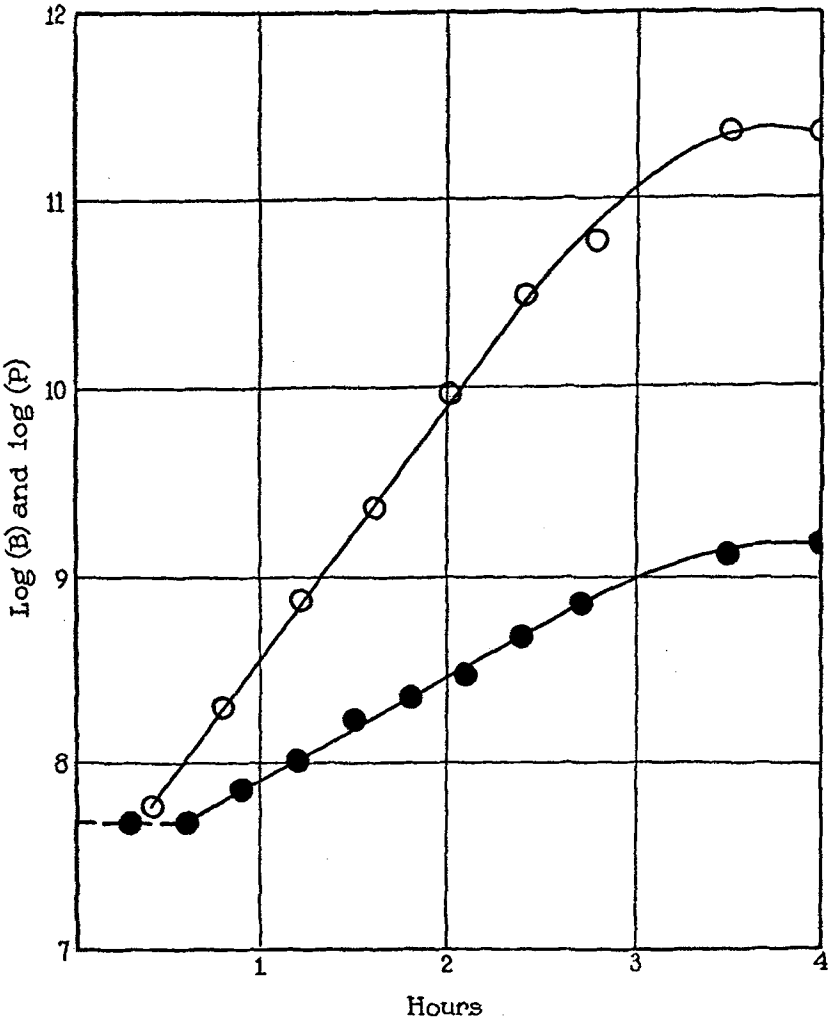

FIG. 3. Temperature 40°C.

At 45°C. the phase of logarithmic bacterial growth drops to a 3-fold increase per hour while phage concentration instead of increasing definitely decreases.

2. Effect of pH on the Phage-Bacterium Reaction

Figs. 5–9 illustrate graphically the influence of H-ion concentrations from 6 to 8.5 on phage production and bacterial growth at 36°C. It is evident that with increasing concentrations of H-ions the lag phase of bacterial growth is prolonged, although the rate of logarithmic

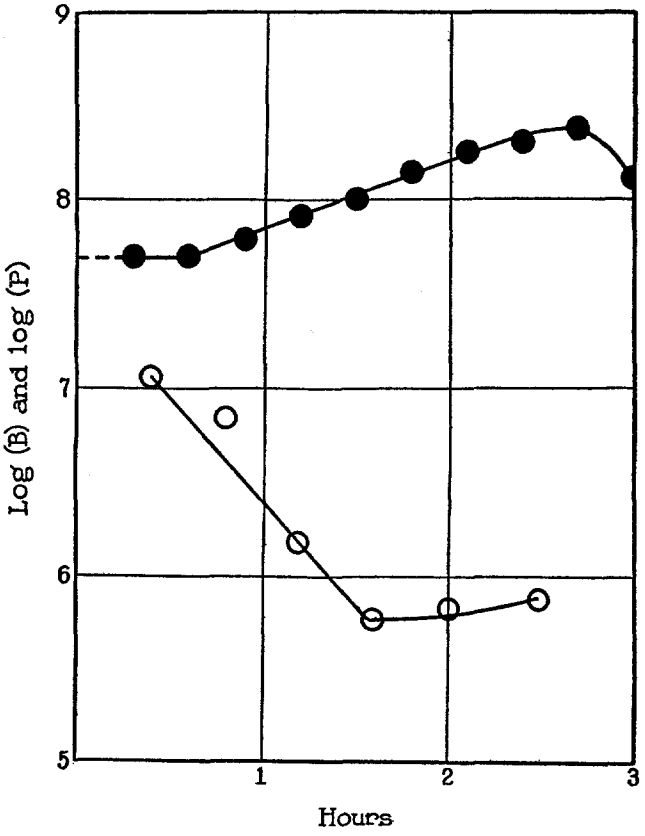

FIG. 4. Temperature 45°C.

increase when once begun is not appreciably altered. Likewise, the rate of phage production is not changed at lower pH's. While a change in pH from 7 to 6 results in increasing the lag phase of bacterial growth from 0.25 hour to 1.25 hours there is no corresponding increase in the lag phase of phage production. As the H-ion concentration is

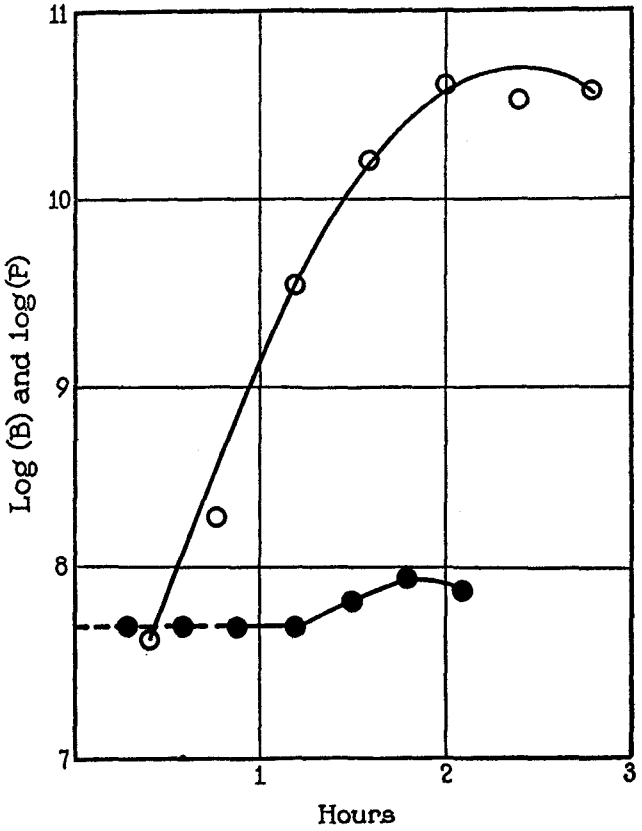

FIG. 5. pH 6

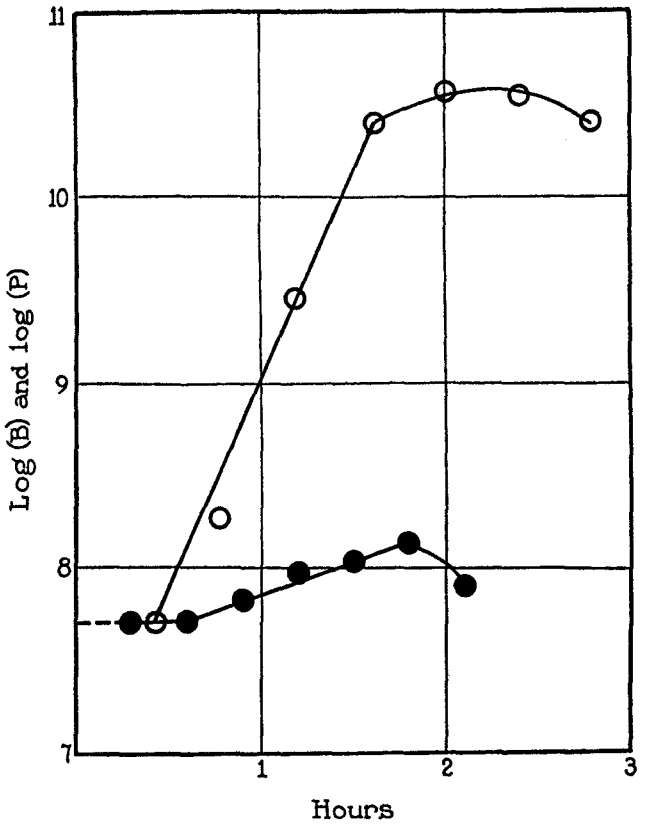

FIG. 6. pH 6.5

FIGS. 5–9. Effect of pH on the phage-bacterium reaction. Phage production and bacterial growth at 36°C. and at various pH values are plotted against time. ○ = phage activity units/ml. ● = bacteria per ml.

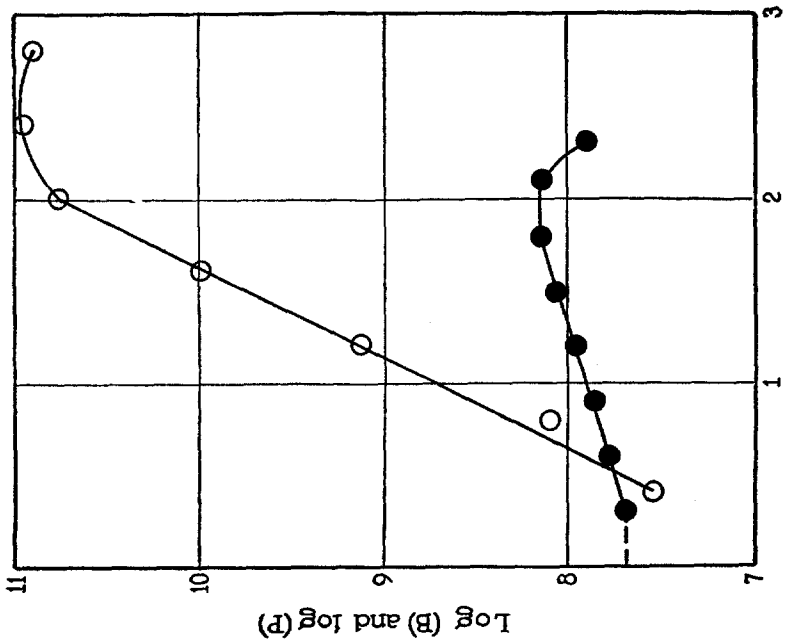

FIG. 8. pH 8

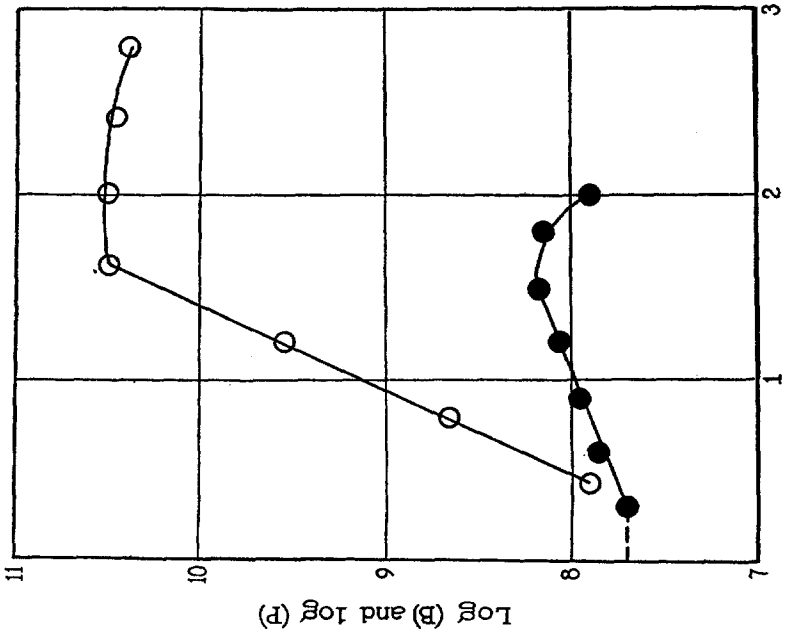

FIG. 7. pH 7

decreased the only noticeable change is a slight drop in the rate of bacterial reproduction occurring at pH 8.5; it is not accompanied by any alteration in the rate of phage production.

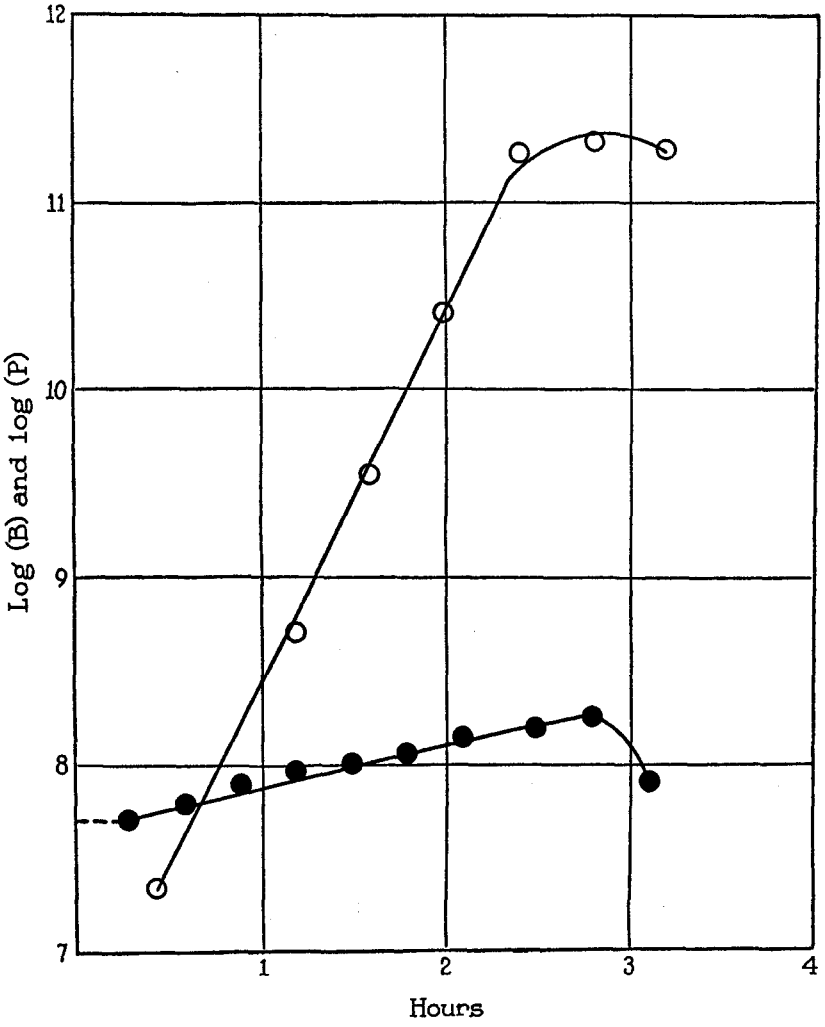

FIG. 9. pH 8.5

In the experiments performed at pH 6 and 36°C. [phage] increased over 10-fold before there was any detectable increase in [bacteria].

To see if this apparent dissociation between the processes of bacterial reproduction and phage formation could be made more evident several identical experiments were carried out at pH 6 with the temperature adjusted to 28°C. instead of 36°C. In Fig. 10 the general results are

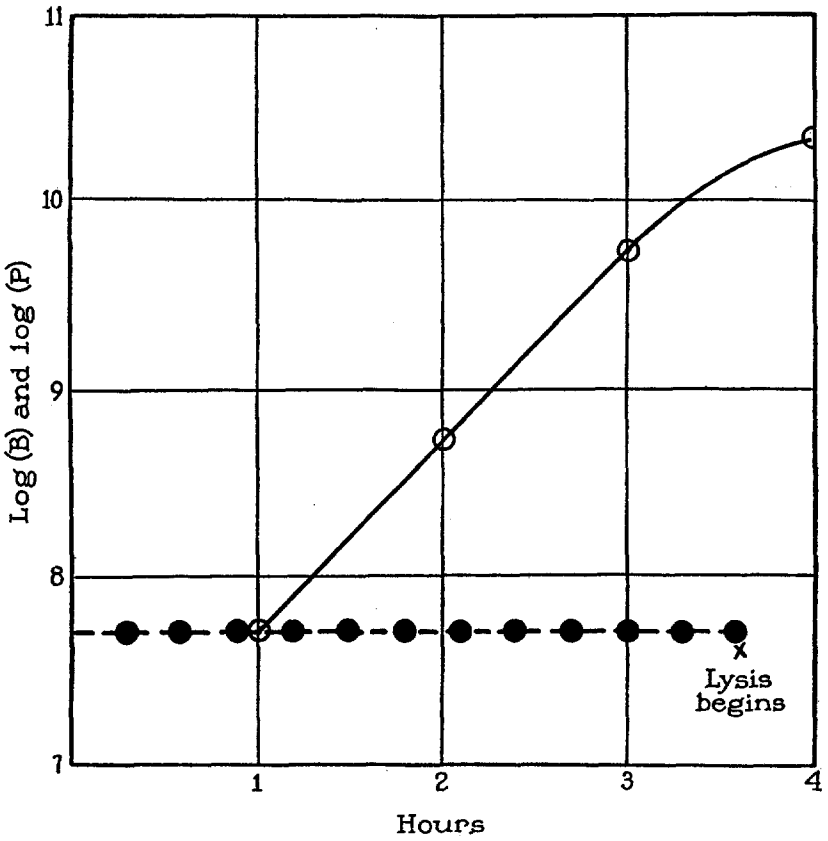

FIG. 10. Phage production in the absence of bacterial growth. [Phage] shows a 10-fold increase per hour while the bacteria show no growth at all. O = phage activity units/ml. ● = bacteria per ml.

plotted and it is clear that under these conditions no observable increase in [bacteria] occurs while [phage] rises over 100-fold.

DISCUSSION

From the data of the experiments performed at different temperatures, it follows that the processes of bacterial reproduction and phage

formation are not related in the simple way previously reported. The equation used by Krueger and Northrop (1) to express phage production in terms of bacterial growth stated that:

$$P/P_o = (B/B_o)^n$$

Where B = bacterial concentration at any time, B_o = initial bacterial concentration, P = phage concentration at any time, and P_o = initial phage concentration. This equation fits the data for which it was derived quite satisfactorily but in the experiments here reported it does not apply. That is, while the rate of bacterial growth shows a regular increment with increasing temperature up to 40°C., phage production exhibits a marked rise in rate as the temperature is raised from 30°C. to 35°C. but at 40°C. the rate of phage formation drops markedly. At this last temperature the time of lysis is delayed. With a further rise in temperature to 45°C. the logarithmic phase of bacterial growth shows a 3-fold increase in population per hour, a somewhat lower rate than that observed at 40°C. The concentration of phage in the mixture instead of increasing definitely diminishes. This may be ascribed to one of two mechanisms. First: it is evident that 45°C. is beyond the optimum temperature for growth of this particular staphylococcus and it is likely that a good many dead cells are accumulating in the suspension. Dead bacteria adsorb phage irreversibly in quite large amounts (6) and the drop in phage may well be due to such adsorption. Second: the loss of phage may be attributed to direct heat inactivation. From the previously reported data of Krueger (7) it is known that this phage does not become inactivated at any such rate until higher temperatures are employed. The first mechanism, namely, removal of phage by adsorption to dead cells seems the most probable one, for after a pronounced drop in [phage] there follows a phase of slow increase in [phage] as might be expected after the available dead cell surfaces had become saturated.

The main feature of the temperature experiments is the fact that the rate of phage production shows an optimum in the neighborhood of 35°C. and the bacterial growth optimum is about 40°C. While the rate of bacterial growth rises as the temperature is raised from 35°C. to 40°C. the rate of phage formation exhibits a pronounced drop.

In the experiments on the effects of H-ion concentration on the

phage-bacterium reaction the most marked differences are observed in the acid range. As [H-ion] increases the lag phase of bacterial growth is increased, although the slope of the phase of logarithmic growth is not appreciably altered when once begun. At pH 6.0 bacterial growth occurs for only 0.6 hour as compared with the logarithmic growth phase of 1.2 hours at pH 7. Likewise, only one-half the total number of cells is produced at pH 6 as at pH 7. Nevertheless, there is no corresponding increase in the lag phase of phage production nor is the rate of phage formation altered by lower pH's. The total amount of phage formed at pH 6.0 and pH 6.5 is identical with that produced at pH 7.0.

With increasing alkalinity there appears to be no pronounced deviation from the normal curves of bacterial growth and phage formation. At pH 8.5 lysis is delayed almost an hour, and the lytic threshold is in the neighborhood of 1000 activity units per bacterium instead of 100–140 as is usually the case.

The pH experiments indicate that phage formation can occur at the normal rate in the absence of bacterial growth (see Fig. 5). In another experiment at pH 6 using a temperature of 28°C. instead of 36°C. we found that phage formation goes on at the rate of a 10-fold increase per hour, while there is no bacterial growth at all during the entire experimental period.

From the above considerations it would appear that the significance previously accorded bacterial growth as the essential conditioning factor for phage production may be questioned. At pH 7.4 and 36°C. the equation expressing phage production in terms of the rate of bacterial growth fits the observed experimental data very well (1). The reason for this good agreement is probably to be found in the fact that both the phage-forming mechanism and the mechanism of bacterial growth happen to follow the course of autocatalytic reactions. The apparent relationship is further emphasized by the cessation of phage formation when bacterial growth is stopped abruptly by altering environmental conditions, *e.g.* shifting to very low temperatures as was done by Krueger and Northrop (1). The dependence of phage production on bacterial growth is, however, more apparent than real, for the two phenomena can be dissociated. When bacterial reproduction is inhibited by the use of broth adjusted to

pH 6 and 28°C. phage production nevertheless proceeds at a rapid rate (Fig. 10). The selection of bacterial growth data for use in deriving the equation for phage production was then merely fortuitous and basically without significance. In place of the expression for bacterial growth there should be substituted the terms for some other reaction which proceeds logarithmically with time but whose nature is at present unknown. This reaction may well consist of the production of a phage precursor which is promptly converted into phage by phage itself. Numerous unpublished observations made in this laboratory over the past 3 years have shown that 100 per cent increases in phage titre can be obtained by adding certain cell-free ultrafiltrates of bacterial preparations of phage.

There is good reason to believe that phage is a protein with the properties of an enzyme (7–11). We feel that the experiments reported in the present paper support this concept, and suggest that the mechanism of phage production can be studied like any other cellular mechanism of enzyme formation under conditions which set it apart from the complexities of cellular growth.

SUMMARY AND CONCLUSIONS

1. The effects of temperature and H-ion concentration on the reaction between antistaphylococcus phage and a susceptible staphylococcus have been studied.

2. The temperature optimum for phage production is in the neighborhood of 35°C. and that for bacterial growth is approximately 40°C.

3. With increasing H-ion concentrations there occur: (*a*) an increase in the lag phase of bacterial growth without any corresponding increase in the lag phase of phage production; (*b*) a diminution in the total bacterial population accumulating in the medium without any corresponding drop in the total amount of phage formed.

4. With increasing alkalinity there is no pronounced change in the curves of bacterial growth and phage formation. At pH 8.5 the lytic threshold is increased to about 1000 phage units per bacterium instead of 100–140 as is usually the case and the time of lysis is delayed.

5. By adjusting the medium to pH 6 and 28°C. bacterial growth can be completely inhibited while phage production continues at a rapid rate.

6. Apparently, the previously stressed importance of bacterial growth as the prime conditioning factor for phage formation does not hold, for under certain experimental conditions the two mechanisms can be dissociated.

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