# STUDIES ON ACTIVATION OF T4 BACTERIOPHAGE BY COFACTOR

## IV. NASCENT ACTIVITY*

by

ELIE L. WOLLMAN** AND GUNTHER S. STENT***

*Service de Physiologie Microbienne, Institut Pasteur, Paris (France)*

### INTRODUCTION

Previous papers in this series have been concerned with the study of cofactor activation of T4 bacteriophages, which require the presence of certain amino acids for adsorption to receptive host bacteria. In particular, the concept of the degree of activity as an expression of the extent to which cofactor has reacted with the phage[1], the kinetics with which a phage population gains or loses this degree of activity[2], and the effect of temperature[3] were considered. A model of the activation mechanism was proposed in order to unify our observations.

The present study attempts to elucidate a phenomenon described by T. F. ANDERSON in early work on this problem[4]. When T4 bacteriophages are plated on cofactor-free agar seeded with receptive bacteria, only one out of $10^4$ to $10^6$ form plaques, whereas *full* plaque counts can be obtained if the same phages are first adsorbed to bacteria in the presence of cofactor before being plated on cofactor-free agar. Having shown that the cofactor requirement is a heritable property of a phage particle and that phages isolated from plaques formed by infected bacteria on cofactor-free agar have the same cofactor requirement as the parental types, ANDERSON stated concerning this surprising observation[5]: "How are the virus particles liberated from these cells activated to carry the infection to other cells in the neighborhood? Only 30 to 40 virus particles are liberated (per cell) from B (bacteria) on F (synthetic) medium. One might estimate their chances of continuing the infection to one of the surrounding cells to be only 30 to 40 times $10^{-6}$. The chances that a chain of such highly improbable reactions would proceed to the destruction of enough bacteria to make a visible plaque would be infinitesimal indeed. We can only surmise that on lysis the complex liberates cofactor sufficient in activity to permit an appreciable fraction of the liberated virus to infect neighboring cells."

It will be shown here that the first generation progeny of cofactor requiring bacteriophages are able to transmit the infection to neighboring bacteria on cofactor-free plates because they possess an activity which is lost only very slowly compared to

---

* This work was begun at the California Institute of Technology during the years 1948–1950.

** Fellow of the Rockefeller Foundation, 1948–1950.

*** Fellow of the American Cancer Society, upon Recommendation of the Committee on Growth of the National Research Council.

*References p. 550.*

tryptophan induced activity of the parental types. We will refer to such phages as *nascent* and to the activity which they possess as *nascent activity*. In contradistinction, the phages of the original T4.38 stock, which require the addition of cofactor for activation, will be described as *quiescent*. Immediately after their liberation from the lysed bacterium, nascent phages degrade towards the quiescent state.

This paper describes experiments designed to follow the kinetics of the process of degradation of nascent into quiescent phages. The low rate of loss of activity of nascent phage in the absence of any external cofactor has made it possible to approach again some problems concerning the nature of cofactor activity, which could not be solved by study of quiescent phage.

## MATERIAL AND METHODS

T4 bacteriophage (stock T4.38), *E. coli* (strain B), and the media F (lactate) and N (nutrient broth) used in this work have been described in a previous publication[1]. The stock T4.38 gives an assay of $10^{10}$ particles/ml when plated on N agar as compared to an assay of only $10^5$ particles/ml when plated on F agar (F/N count = $10^{-5}$).

Adsorption and deactivation experiments were carried out in a non-nutrient medium, referred to as F–L, having the same composition as F medium, but containing no lactate.

The expression *standard bacterial suspension* refers to a twenty-fold dilution in F–L medium of a washed and resuspended 24 h culture of *E. coli* (grown in F medium). Such a suspension contains approximately $2.5 \cdot 10^8$ viable cells/ml.

## DEMONSTRATION OF NASCENT ACTIVITY

Plaque propagation on F agar by a bacterium infected with a cofactor-requiring phage might, as suggested by ANDERSON, be due to the liberation of cofactor upon lysis of the host cell. An experiment was designed to test the possibility whether adsorption of T4.38 to receptive bacteria is possible in the immediate neighborhood of bacteria lysed by another phage. Parallel platings of mixtures of T4.38 and increasing quantities of T1 phages were made on F and N agar plates, seeded for this purpose with a mixture of B/1.5 and B/4 bacteria.

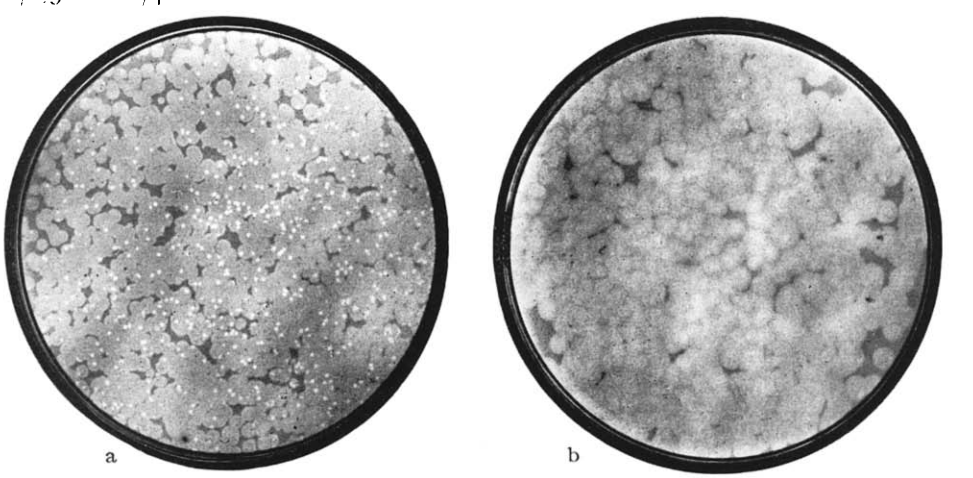


Fig. 1. A mixture of T4.38 (small plaques) and T1 (large plaques) bacteriophages plated with B/4 and B/1 mixed indicators.
a. On nutrient broth agar (N); Both types of plaques appear.
b. On synthetic agar (F); Only T1 plaques visible.

*References p. 550.*

Two plates of this experiment, on which enough T1 was plated to give almost confluent lysis of B/4 bacteria, are presented in Fig. 1a and Fig. 1b. It is seen that within the large plaques due to the action of T1 on B/4, the small plaques due to the action of T4.38 on B/1,5 appear only on the N agar but not on the F agar plates. Neither did any T4.38 plaques appear on any F agar plates on which a much greater number of T1 phages had been plated than on that shown in Fig. 1b. Almost every T4 phage on the F agar must have been in a region where many B/4 cells had been lysed but was, nevertheless, unable to infect neighboring B/1,5 bacteria. If the by-products of lysis of B/4 by T1 do not differ from those released by lysis of B by T4, it would then appear that bacterial lysis does not liberate cofactor in sufficient quantity to allow activation of cofactor-requiring phage.

It is likely, therefore, that phage is already in an active state when it is released from the bacterium. This inference was examined by means of a one-step growth experiment, in which a bacterial culture growing in F medium was infected with T4.38 phage and incubated at 37° C after further dilution into tryptophan-free medium. Platings from this diluted culture were then made at regular time intervals on F and N agar plates.

The result of this experiment is presented in Fig. 2, where the logarithm of the number of infective centers per unit volume is plotted against the time elapsed since infection. It is seen that the platings on N agar exhibit the normal one-step growth behaviour of T4 in F medium: a latent period of 28 minutes is followed by a rise period of 15 minutes, during which the titer increases by a factor of 45. The number of plaques found on F agar is equal to that on N agar during the latent period because each infected bacterium registers as an infective center on both types of agar. At the onset of lysis the number of plaques formed on F increases until at the end of the rise period it has attained a value ten times higher than the initial titer, but lower than that on N plates. Thereafter whereas the titer as measured on N agar remains constant, the number of plaques formed on F continuously decreases at such a rate that it has been reduced again by a factor of ten thirty minutes later.

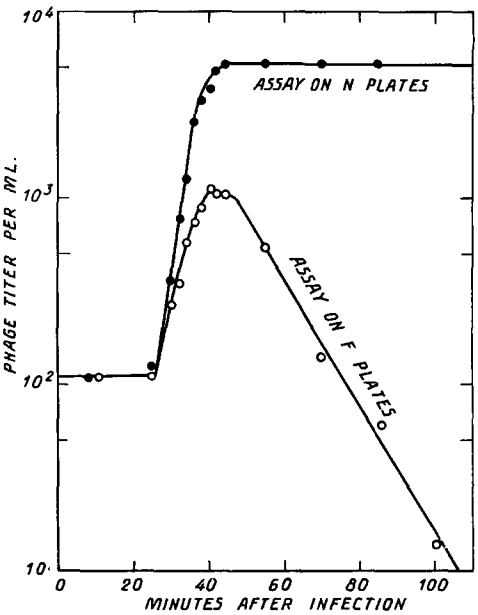


Fig. 2. One step growth experiment of T4.38 phage in F medium with assays on N and F agar.

The rise in titer on F plates observed at the end of the latent period can only mean that *the newly released nascent phages issue from the host cells in an active state.* For had phage been liberated in an *inactive* state, the number of plaques registered on F agar should have decreased at the end of the latent period due to the disappearance of infected bacteria, at the same time as the titer registered on N agar increased. The failure of the number of plaques on F agar to equal that on N agar at the end of the

*References p. 550.*

rise period as well as the subsequent decrease in the titer measured on F agar are manifestations of the transient nature of this nascent activity.

The properties of nascent phages and the nature of nascent activity are examined in the following and an attempt will be made to interpret our findings in terms of the model of cofactor activation of quiescent phages already proposed[2].

## PROPERTIES OF NASCENT PHAGE

### *Preparation*

Due to the transient nature of nascent activity it is necessary to examine the nascent phages immediately after their release during a one-step growth experiment. For the experiments described below, a so-called *fresh suspension of nascent phage* was always prepared in the following manner:

T4.38 bacteriophage, pre-equilibrated with tryptophan, is added to a washed suspension in F–L medium of bacteria removed in their exponential growth phase from F medium. This adsorption mixture contains $2 \cdot 10^8$ phages/ml, $5 \cdot 10^8$ bacteria/ml and 2 mmg/ml tryptophan. After 5 minutes, 90% of the phages have been adsorbed; the mixture is then diluted one-hundred fold into F medium and incubated at 37° C. 44 minutes after infection, when lysis of the infected bacteria is complete, the lysate is chilled in ice, $10^9$ heat-killed B/4 bacteria per ml are added as carrier, and the mixture centrifuged in the cold to eliminate from the lysate any remaining bacteria sensitive to T4. The titer of such a fresh suspension is $10^8$ particles/ml when plated for assay on N agar and $10^7$ particles/ml on F agar.

### *Loss of activity*

To study the rate of loss of nascent activity, aliquots of a fresh suspension of nascent phage diluted in F–L medium were incubated at 37, 26 and 15° C and platings made on F and N agar after various time intervals. The results are presented in Fig. 3 where the logarithm of the ratio of corresponding assays on both types of agar, referred to as the F/N count, is plotted against the time of incubation. It is seen that nascent activity is lost in a roughly first-order manner, but with very different velocities at the three temperatures, corresponding to rate constants of 0.07 (37° C), 0.029 (26° C) and 0.016 (15° C) per minute.

On Fig. 3 is also presented the rate of loss of activity of tryptophan-activated quiescent phage at 15° C, as measured by the techniques described previously[1,2]. It is apparent that this deactivation rate, corresponding to a rate constant of 0.46 per minute, is very much greater than that of nascent phages at the same temperature. It may be concluded, therefore, that *nascent activity cannot be solely due to the acquisition by the phage particles before lysis of a tryptophan-like cofactor* (*i.e.* one having an affinity for the phage similar to that of tryptophan).

In subsequent experiments we will take advantage of the extremely slow deactivation of nascent phage at low temperatures and carry out some dilutions of nascent phage in cooled medium to arrest loss of activity at a given time.

### *Adsorption rates*

The F/N count of even the freshest suspension of nascent phage does not exceed 0.2. Does this fraction represent a part of the phage population capable of forming plaques on F agar while some 80% of the phages are completely inactive, or is it the outcome of a competition between loss of nascent activity and adsorption on the plate in which the entire population participates? Since the factors governing plaque formation on

*References p. 550.*

agar are not sufficiently well known, it is difficult to form a quantitative interpretation of nascent activity on the basis of the F/N count.

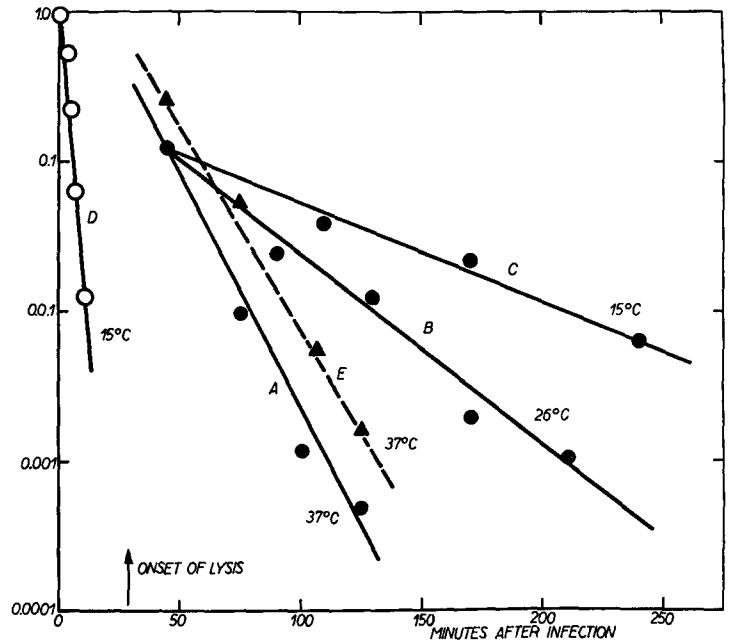


Fig. 3. Deactivation of nascent phage. F/N counts of nascent phage suspensions at various times after the infection of the original bacterial culture: Incubation at 37° C: Curve A; Incubation at 26° C: Curve B; Incubation at 15° C: Curve C. Degree of activity of quiescent phage after dilution of tryptophan: Incubation at 15° C: Curve D. Degree of activity of nascent phage, as measured by rate of adsorption. Incubation at 37° C: Curve E.

In our previous work the extent of cofactor-induced activity of quiescent phage had been estimated by measurements of adsorption rates in liquid medium[1]. On this basis, the *degree of activity* of a phage population had been defined as the ratio of the rate constant of adsorption in synthetic medium supplemented with a certain cofactor concentration to that in broth. In order to extend this definition to nascent activity, we have compared the rates of adsorption of a fresh nascent phage suspension in F–L medium and in broth.

*Procedure*

A fresh suspension of nascent phage is added to a standard bacterial suspension at 15° C. At various times aliquots are diluted into an ice-cold suspension of resistant B/4 bacteria to stop adsorption. This mixture is centrifuged and the pellet resuspended after two washings in ice-cold F–L medium. The fraction of the phage input adsorbed at the time of dilution is estimated from the ratio of assays made before and after centrifugation.

A parallel adsorption experiment is carried out with a standard bacterial suspension in nutrient broth.

The results of such an adsorption experiment performed at the temperature of 15° C, at which the rates of adsorption of quiescent phages had been measured, are presented in Fig. 4a, where the fraction of input fresh nascent phage adsorbed is plotted

*References p. 550.*

against the time of contact of phage with a standard bacterial suspension in broth and in F–L medium. It is found that in F–L medium, fresh nascent phage is adsorbed exceedingly slowly, at a rate of only 0.015 of that in broth, and hence the degree of activity at 15° C of fresh nascent phage is no more than 0.015.

It is surprising in view of this rather low degree of activity of nascent phage that plaques on F agar are nevertheless formed with an efficiency of 20%. Since the plates are incubated at 37° C, the rate of adsorption of nascent phage was measured also at this temperature. Fig. 4b presents the result of this experiment, where the rate of adsorption at 37° C in broth and in F–L medium are shown. It is apparent that at 37° C the adsorbability of nascent phage is much more similar in the two media than at 15° C, the ratio of the rate constant of adsorption in F–L medium to that in broth now corresponding to a degree of activity of 0.25.

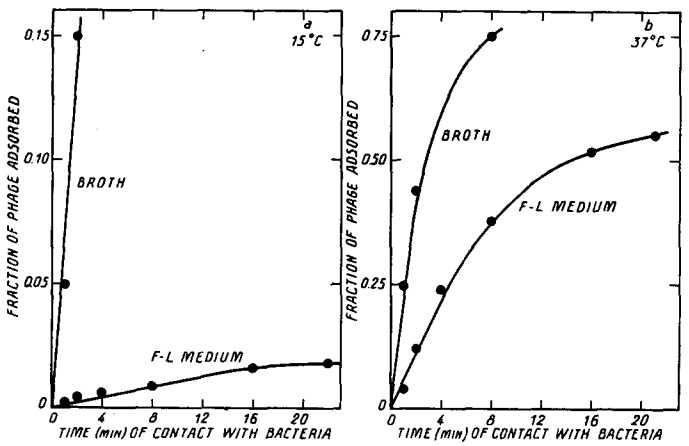


Fig. 4. The rate of adsorption of fresh nascent phage to a standard bacterial suspension ($2.5 \cdot 10^8$ cells/ml) in F–L medium and in broth. a. at 15° C; b. at 37° C.

If these observations are compared with the tryptophan-induced activity of quiescent phage, it is seen that this striking increase of the degree of activity of nascent phage with temperature is very similar to the temperature sensitivity of the degree of activity of quiescent phage. A fresh suspension of nascent phage is adsorbed in cofactor-free medium at both 15° C and 37° C with rates like quiescent phage suspended in medium containing 0.5 to 0.7 mmg/ml tryptophan. A discussion of the implications of this similarity must be deferred until a later section of this study.

*All-or-none and intermediate-state theories*

To extend the meaning of the degree of activity to *individual* phage particles of a population of cofactor-activited quiescent phages, two principal alternatives of the manner in which a phage particle can become "activated" had been considered. Under the *all-or-none* theory, phages are either fully active or inactive, and the degree of activity indicates the fraction of the phages in the active state. Under the *intermediate state* theory, each particle may pass through a spectrum of states intermediate to the extremes of the all-or-none theory, and the degree of activity measures the property of a state of the members of the population. For methodological reasons, it had not been

*References p. 550.*

possible to decide between the two alternatives. Because of their high degree of activity at 37° C in the absence of any external cofactor, a similar analysis of the degree of activity can now be applied successfully to a population of nascent phages. If the all-or-none theory were true, then, at the degree of activity of 0.25 observed for nascent phage at 37° C, it should never be possible to adsorb more than this fraction of the population in cofactor-free medium. Actually, as may be seen from Fig. 4b, sixty per cent. of the phage input of a fresh nascent phage suspension can be adsorbed. In additional experiments concerning this point it was found that if a bacterial concentration four times that of the standard suspension is employed for adsorption, no less than ninety per cent. of fresh nascent phages can be adsorbed in cofactor-free minimal medium. Hence it is apparent that practically the entire fresh population is potentially adsorbable. The overall rate of adsorption of only one fourth that of maximally active phage must therefore be due to the fact that individual phage particles are in some intermediate state between inactivity and maximal adsorbability.

*Degree of activity in the course of deactivation*

Loss of activity of a nascent phage population, such as that presented in Fig. 3, may now be interpreted as a continuous degradation of all members of the population into states of lower and lower adsorbability. In order to see to what extent the decreasing F/N count reflects a change in degree of activity during deactivation, the adsorbability of a nascent phage suspension was tested after incubation at 37° C for various lengths of time. The results of this experiment are presented as curve E in Fig. 3, the logarithm of the degree of activity, as estimated by the ratio of the rate constants of adsorption at 37° C, having been plotted there against the time after infection of the parent culture at which the rate of adsorption of the deactivating phage was measured. It is seen that degree of activity and F/N count decrease in parallel at the same temperature. Hence the F/N count is a relative measure of the degree of activity of a nascent phage population. Direct plating of nascent phage on minimal F agar plates thus appears to be analogous to the "dump experiment" previously described with quiescent phage, the agar here being the medium of the adsorption-deactivation competition.

A fresh suspension of nascent phage is constituted of particles which were liberated from the lysed bacteria at different times during a 15 minutes interval (rise period), a time sufficiently long to permit a considerable deactivation at 37° C of the first particles liberated. Accordingly, it may be expected that the fresh suspension is itself a mixture of particles of different degrees of activity. An experiment was designed to reveal this heterogeneity.

*Procedure*

Aliquots of a fresh nascent phage suspension are mixed with two bacterial suspensions, one containing $10^9$ (Tube I) and the other $2.5 \cdot 10^8$ (Tube II) bacteria/ml. Both mixtures were centrifuged in the cold after incubation at 37° C for 4 minutes, when 44 and 18 % of the phage input respectively had been adsorbed. The rates of adsorption of the free phages remaining in the supernatant of each aliquot were then measured and compared with that of a third, unfractionated aliquot (Tube III) of the original suspension incubated in the absence of bacteria for the same length of time as tubes I and II.

Table I presents the results of this experiment, from which it is seen that the greater the fraction of input phage adsorbed, the less adsorbable in the unadsorbed

*References p. 550.*

remainder, illustrating the suspected heterogeneity of the nascent phage suspension. The observed difference in adsorbability of different fractions examined appears to be greater than that one might anticipate on the basis of the deactivation rates, suggesting that the phages are already released from the bacterium in different states of activity.

TABLE I

FRACTIONATION OF FRESH NASCENT PHAGE SUSPENSION

| *Tube* | *Bacterial concentration cells/ml* | *Fraction remaining unadsorbed after incubation at 37°C for 4 minutes* | *Adsorption rate constant of remainder* $\times 10^{11}$ *ml/min* |
|---|---|---|---|
| I | $10^9$ | 0.56 | 3 |
| II | $2.5 \cdot 10^8$ | 0.82 | 6 |
| III | 0 | 1.00 | 14 |

## NATURE OF NASCENT ACTIVITY

It was inferred from the great difference in rates of deactivation between nascent phages and tryptophan-activated quiescent phages, that a tryptophan-like cofactor acquired by the phage before lysis could not be the sole source of its nascent activity. In considering further the nature of nascent activity three main hypotheses may be considered:

1. *Special cofactor.* Nascent activity may be due to a *special cofactor* which the phages have acquired inside the bacterium and which possesses a much greater affinity for the bacteriophage surface than tryptophan. Loss of nascent activity and degradation towards quiescent phage would then be due to the loss of this special cofactor.

2. *Special surface.* Nascent phage may differ from quiescent phage in the property of its *surface*. This special surface would be so constituted that while intact it resembles that of non-cofactor requiring phages, promoting adsorption in the absence of *any* cofactor. Loss of nascent activity would be equivalent to a gradual change of this surface. (The operational distinction between this and the preceding alternative rests largely on the possibility of isolating or preparing a "special" cofactor).

3. *Tryptophan-like cofactor—special surface.* Nascent activity could be a combination of two effects: the *activity* possessed by the phages arising from activation by a tryptophan-like cofactor acquired inside the bacterium, and the *stability* of this activity being due to the increased affinity of the cofactor because of the presence of a special nascent surface. In the course of degradation of nascent into quiescent phage, both cofactor and surface would be lost to the particles.

### *Deactivation of tryptophan-reactivated nascent phage*

To gain further insight into the nature of nascent activity, suspensions of nascent phage which had lost their activity to different degrees were *reactivated* by the addition of tryptophan. The rate of loss of the *tryptophan-induced activity* was then examined. Were degradation of nascent activity due to the loss of special cofactor or surface, the induced activity should disappear in this experiment at a rate characteristic of the deactivation of tryptophan-activated quiescent phage. If, on the other hand, slow loss

*References p. 550.*

of tryptophan-like cofactor was primarily responsible for disappearance of nascent activity, some of the activity induced by tryptophan in this experiment should be more stable than that imparted to quiescent phage.

*Procedure*

Two aliquots of a fresh nascent phage suspension are incubated at 37° C (Tube A) and 15° C (Tube C) and intermittent platings are made on F and N agar. Samples are taken from tube A at three different times and reactivated at 37° C by addition of 2 mmg/ml tryptophan (activation mixtures). After a short time has been allowed for activation, the activation mixtures are diluted in F–L medium at 15° C to a residual tryptophan concentration of 0.1 mmg/ml (deactivation mixtures). From the deactivation mixtures incubated at 15° C intermittent platings are made on F and N agar.

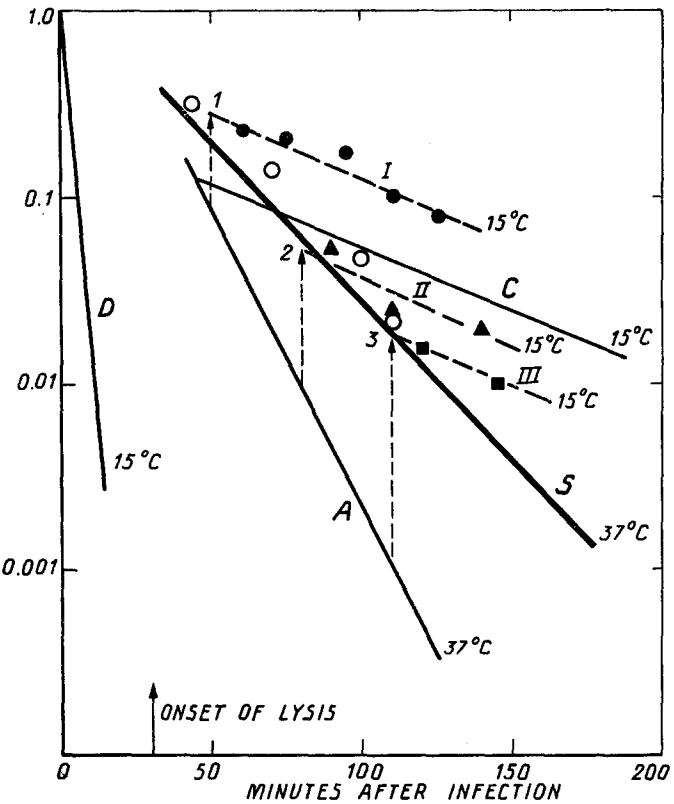


Fig. 5. Deactivation of nascent phage reactivated by tryptophan. F/N Count of nascent phage suspensions at various times after the infection of the original bacterial culture. Incubation at 37° C: Curve A; Incubation at 15° C: Curve C; Incubation at 37° C in the presence of 2 mmg/ml tryptophan: Points (o); Incubation at 37° C, reactivation with 2 mmg/ml tryptophan at time indicated by arrow, then incubation in absence of tryptophan at 15° C: Curves I (●), II (▲); III (■). Degree of activity of quiescent phage after dilution of tryptophan: Incubation at 15° C: Curve D. Loss of nascent surface: points 1, 2, 3; curve S.

The results of this experiment are presented in Fig. 5, where the logarithm of the F/N count is plotted against the time elapsed between infection of the original bacterial culture and plating. Curves A and C represent the loss of nascent activity at 37° C and 15° C with their characteristic rates (platings from tubes A and C). Curve D indicates the typical rate of loss of tryptophan-induced activity of quiescent phage, as determined by methods previously described[2]. The points along curves I, II and III represent F/N counts obtained by platings from the deactivation mixtures. The time at which each deactivation mixture had been prepared by tryptophan addition to and subsequent removal from an aliquot of tube A is indicated by a vertical arrow.

It is to be seen, first of all, that the F/N counts obtained by plating the reactivated

*References p. 559.*

nascent phage are considerably higher after than before reactivation. The increased activity, furthermore, is lost at a rate characteristic of the loss of nascent activity, as is evident from the parallelism with curve C of curves I, II and III. Had the interaction between nascent phage and tryptophan been of the same kind as cofactor activation of quiescent phage, any activity imparted to phage thereby should have been lost again quickly at the rate indicated by curve D until curve A was again reached.

The nature of nascent activity appears to correspond therefore to the third alternative discussed above, *i.e.* to be due to a combination of a tryptophan-like cofactor and a special surface responsible for its higher affinity.

At the time of reactivation there must have been present in the phage population a considerable fraction of the special surface which was not covered by cofactor and to which the added tryptophan attached itself. The loss of nascent activity is then to be thought of as a slow loss of an ordinary cofactor.

It is to be noted that the highest F/N count to which an aging nascent phage suspension can be reactivated also decreases with time, as evidenced by the points 1, 2 and 3, being respectively the initial points of curves I, II and III. This observation is to be interpreted as a manifestation of loss of the special surface.

Old stocks of cofactor-requiring phage, like our T4.38 stock, are quiescent even though they may have been made and stored in nutrient broth, showing that the presence of cofactor does not prevent the degradation of nascent into quiescent phage. To estimate more directly the rate of loss of special *nascent surface*—already suggested by points 1, 2 and 3, the deactivation of a fresh nascent phage suspension was followed at 37° C in the presence of 2 mmg/ml of tryptophan. Dilutions of this suspension were made at certain intervals into F–L medium and allowed to stand at 15° C for 10 minutes a length of time during which all tryptophan-induced activity not connected with nascent surface would be lost while all nascent activity would be preserved. Platings were then made on F and N agar.

The results of this experiment are also presented on Fig. 5 as hollow circles. It is seen that these points lie well above curve A, representing the deactivation of nascent phage in the absence of tryptophan at the same temperature. A straight line S may be fitted to these points, which also connects 1, 2 and 3, the levels to which aged nascent phage could be reactivated at various times. The line S then represents the loss of the special surface of nascent phage, and since its slope is somewhat less than that of curve A, it may be concluded that during deactivation of nascent phage *cofactor is lost more rapidly than the special surface* for which it has a great affinity.

## DISCUSSION

### *Mechanism of nascent activation*

We may now consider the principal features of nascent activity in the light of the model devised to interpret cofactor activation of quiescent phage[2]. Phage particles were thought to contain *key-sites* which exist in physiologically active or inactive states, the degree of activity of a phage population being equal to the fraction of these key-sites in the active state. Single cofactor molecules adsorb or desorb rapidly at these sites, an equilibrium constant ($K_c$) expressing the position of the adsorption-desorption equilibrium. At those key sites at which five or more such cofactor molecules are adsorbed together, a reaction takes place which activates the site and binds the cofactor molecules.

*References p. 550.*

Deactivation of the site occurs when the complex of five cofactor molecules breaks up with a certain probability ($k_d$) per time unit.

The distinguishing feature of nascent phage is the slowness with which it loses tryptophan-induced activity. We attributed this greater affinity for cofactor to the presence of a special nascent surface. In terms of the model of cofactor activation, it may now be stated that after cofactor has been removed from the medium, the key-sites of nascent phage remain longer in the active state than the key-sites of quiescent phage. This could be due to a modification of the *properties* of the key-sites under the influence of the nascent surface. Another possibility would be that the nascent surface has a great *affinity* for tryptophan, the *activity*, however, still being due to a secondary reaction of cofactor with the otherwise unmodified key-sites.

Under the first alternative, the presence of the nascent surface may be thought to modify one of the interactions between cofactor and key-sites already considered, *i.e.* that its presence may either increase the affinity of free cofactor molecules for the key-site, measured by the equilibrium constant $K_c$, or it may reduce the rate of dissolution of the complex of five cofactor molecules, measured by the rate constant $k_d$. The exponential mode of loss of nascent activity would, in this case, have to be interpreted as an indication that key-sites are either *fully* nascent with high affinity for cofactor, or *fully* quiescent with the $K_c$ or $k_d$ characteristic of that state. During the degradation of nascent into quiescent phage, the *fraction* of all key-sites having the nascent character then decreases according to curve S of Fig. 5. For if the affinity for cofactor of individual key-sites decreased *gradually* as nascent surface disappears, then the loss of activity of an aging suspension of nascent phage should occur with an accelerating rate, finally reaching the rate of deactivation of quiescent phage.

Under the second alternative, the key-sites embedded within the nascent surface, which, so to say, acts as a source of cofactor to them, would then impart an activity to the phage particle related to the amount of cofactor retained by the surface. The loss of nascent activity, under this hypothesis, would be interpreted as leakage of cofactor from the nascent surface which, in turn, would result in a smaller number of key-sites in the active state. The loss of nascent surface represented by curve S of Fig. 5 would be thought to constitute a reduction of the *capacity* of this source.

*Temperature dependence of nascent activity*

It was found in this study that the degree of activity of fresh nascent phage in F–L medium as measured by adsorption rates is similar to that of a suspension of quiescent phage activated by 0.5 to 0.7 mmg/ml tryptophan both at 15° C and 37° C, *i.e.* that the activity of nascent phages has the same high temperature dependence as the activity of quiescent phages exposed to low cofactor concentrations.

By means of experiments in which activation of quiescent phage had been effected at different temperatures but adsorption at only one standard temperature, it had been demonstrated that the great increase in the degree of activity of quiescent phage with temperature was actually due to a *more extensive reaction* between cofactor and phage at the higher temperature[3]. Hence the great temperature dependence of the degree of activity of quiescent phage was explained in terms of the key-site model by considering that at low cofactor concentrations the fraction of key-sites having the necessary five cofactor molecules for activation depends on the fifth power of the equilibrium constant $K_c$ and that any slight temperature increase of $K_c$ would be amplified to its fifth power

*References p. 550.*

in its effect on this fraction. It has since been possible to justify this interpretation further by showing that quiescent phages activated by a low cofactor concentration at one temperature but exposed to bacteria at different temperatures for measurement of their relative adsorbability exhibit the *same* degree of activity.

When the temperature sensitivity of the degree of activity of nascent phage is considered, it must be recalled that the adsorption measurements in F–L medium were always carried out in dilute suspensions devoid of any external cofactor. Hence it is not possible that a more extensive reaction between phage and *medium* cofactor occurred at the higher temperature. Instead, as suggested by the second alternative discussed above, the nascent surface appears to act as a cofactor source for the key-sites, which, in the case of a fresh nascent phage suspension, supplies the key-sites with a cofactor "atmosphere" equivalent to that produced when 0.5 to 0.7 mmg/ml tryptophan are added to the medium. The nascent key-sites react with this "atmosphere" precisely as would quiescent key-sites at both temperatures, in that the fraction of nascent key-sites having the necessary five cofactor molecules also depends on the fifth power of the same equilibrium constant $K_c$.

## CONCLUSION

The ability of cofactor-requiring bacteriophages to form plaques on synthetic medium when already adsorbed to bacteria before being plated has been shown to be due to a transient *nascent activity* of phages just released from infected bacteria. It is inferred that the high affinity of tryptophan-like cofactor for a *special surface* of such phages is responsible for the slow loss of nascent activity. The special surface itself is unstable and its loss constitutes the real degradation of nascent into quiescent phage.

A surface modification of T2 and T4 bacteriophages grown in synthetic medium has been found by S. S. COHEN to be due to a coat of desoxyribonucleic acid amounting to as much as 30% of the total DNA content of these particles[6]. Treatment with desoxyribonuclease removes this coat without affecting the phage titer. It was not possible, however, to establish a connection between this DNA coat and the nascent surface, since addition of desoxyribonuclease to a fresh suspension of nascent phage did not affect the stability of its activity.

The term *nascent phage* was first employed in another connection by EVANS[7], who found that stocks of a certain bacteriophage were inactive on one strain of streptococci but could lyse this strain if a small quantity of a growing *sensitive* streptococcus was added to the resistant strain. EVANS' interpretation of her findings was that bacteriophages at the time of their formation within sensitive cells, *i.e.* nascent phages, are in a "potent" phase which permits them to attack otherwise resistant bacteria. If these conclusions were warranted by more direct evidence, a more than verbal connection might exist between the phenomenon studied by EVANS and the one described in the present work.

The degrees of dependence of phages upon cofactor for adsorption form a series extending from the complete cofactor independence of phages like T2 which are able to adsorb in minimal media at all temperatures, through T4 "temperature mutants" which require cofactor for adsorption only at low temperatures, to the complete dependence of quiescent phages like T4.38. Nascent phage is a new link in this chain, resembling temperature mutants in its properties before it has degraded into quiescent

*References p. 550.*

phage. If we are to envision that cofactor-requiring phages evolved from sufficient phages, then they would appear to retain at a certain phase of their life history the property of the type from which they arose.


## SUMMARY

1. The phages released upon lysis of bacteria infected with a cofactor-requiring strain possess a nascent activity, which makes possible their adsorption in cofactor-free medium.

2. Nascent activity is lost at a rate considerably below that of deactivation of tryptophan-activated T4 (quiescent) bacteriophage. The rate of loss is very temperature-dependent.

3. Individual nascent phages exist in various states of intermediate activity.

4. Fresh nascent phages adsorb very much more slowly in liquid synthetic medium than in broth at 15° C but are adsorbed at nearly equal rates in both media at 37° C.

5. Some of the activity imparted to nascent phages by addition of tryptophan is lost again at the slow rate characteristic of nascent activity. Degradation of nascent into quiescent phage appears to be due to loss of a cofactor as well as to a change in surface.

## RÉSUMÉ

1. Les bactéries infectées par une souche de bactériophage qui ne peut s'adsorber qu'avec l'aide d'un cofacteur, libèrent, après la lyse, des particules possédant une activité naissante qui leur permet de s'adsorber dans un milieu dépourvu de cofacteur.

2. L'activité naissante est perdue à une vitesse considérablement inférieure à celle à laquelle disparaît l'activité conférée par le tryptophane aux bactériophages T4 quiescents. Cette vitesse varie avec la température.

3. Les corpuscules de bactériophages naissants peuvent passer par différents états d'activité relative avant de devenir des bactériophages quiescents.

4. A 15° C les phages naissants s'adsorbent beaucoup plus lentement en milieu synthétique liquide qu'en bouillon, mais les vitesses d'adsorption dans ces deux milieux sont très voisines à 37° C.

5. Une partie de l'activité conférée au phage naissant par addition de tryptophane est ensuite perdue lentement, à la vitesse caractéristique de la perte de l'activité naissante. La transformation du phage naissant en phage quiescent semble être dûe à la fois à la perte d'un cofacteur et à un changement dans les propriétés de sa surface.

## ZUSAMMENFASSUNG

1. Die Bakterien, welche mit einem Bakteriophagenstamm infiziert sind, der nur in Gegenwart eines Kofaktors adsorbiert werden kann, setzen bei der Lyse Teilchen in Freiheit, welche eine nascierende Aktivität besitzen auf Grund derer sie in Kofaktor-freiem Medium adsorbiert werden können.

2. Die nascierende Aktivität verschwindet viel langsamer als diejenige, welche dem ruhenden Bakteriophagen T4 durch Tryptophan verliehen wird. Die Geschwindigkeit des Aktivitätsverlustes hängt in starkem Masse von der Temperatur ab.

3. "Nascierende" Bakteriophagen-Teilchen machen verschiedene Aktivitäts-Stadien durch.

4. Frische nascierende Phagen adsorbieren viel langsamer in flüssigem synthetischen Medium als in Bouillon bei 15°, werden aber in beiden Medien bei 37° ungefähr gleich schnell adsorbiert.

5. Ein Teil der durch Zugabe von Tryptophan an nascierende Phagen verliehenen Aktivität geht langsam wieder verloren und zwar mit der für nascierende Aktivität charakteristischen Geschwindigkeit. Der Übergang des nascierenden Bakteriophagen in den ruhenden Bakteriophagen scheint sowohl dem Verluste eines Kofaktors als einer Obenflächenveränderung zuzuschreiben zu sein.


## REFERENCES


1 E. L. WOLLMAN AND G. S. STENT, *Biochim. Biophys. Acta*, 6 (1950) 292.
2 G. S. STENT AND E. L. WOLLMAN, *Biochim. Biophys. Acta*, 6 (1950) 307.
3 G. S. STENT AND E. L. WOLLMAN, *Biochim. Biophys. Acta*, 6 (1951) 374.
4 T. F. ANDERSON, *J. Cell. Comp. Physiol.*, 25 (1945) 17.
5 T. F. ANDERSON, *J. Bact.*, 55 (1948) 651 and 659.
6 S. S. COHEN, *J. Biol. Chem.*, 168 (1947) 511.
7 A. C. EVANS, *J. Bact.*, 39 (1940) 597.


Received March 7th, 1952