JOURNAL OF VIROLOGY, July 1969, p. 94-108
Vol. 4, No. 1
Copyright © 1969 American Society for Microbiology
Printed in U.S.A.

Effect of Host Cell Wall Material on the Adsorbability of Cofactor-requiring T4[1]

DENNIS T. BROWN[2] AND THOMAS F. ANDERSON
Institute for Cancer Research, Philadelphia, Pennsylvania 19111

Received for publication 7 March 1969

The adsorbability of T4 on host cells was determined as a function of time after their liberation from infected cells. Freshly liberated (nascent) particles are readily adsorbed but lose their adsorbability with a half-time of about 2 days at 5 C, but only about 20 min at 37 C. They can be made adsorbable again with an α-amino acid cofactor like L-tryptophan, and this state of adsorbability can be stabilized by cell wall material from *Escherichia coli.* Such stabilized particles lose their adsorbability at a rate similar to that at which nascent particles lose theirs. Most freshly liberated particles are observed by means of electron microscopy to have "debris" attached to their baseplates and to have most of their six, long tail fibers free, whereas "old" particles that have lost their adsorbability appear relatively "clean" with most of their tail fibers wrapped around their sheaths. Nascent particles have densities that are lower than those of old particles. The material responsible for nascent adsorbability seems to be a fragment of the host's cell wall, for nascent adsorbability is destroyed by lysozyme. Furthermore, nascent T4 particles liberated from host cells with radioactively labeled walls carry the label in density gradients but lose it as they lose adsorbability. In addition, only a small proportion of particles liberated from infected spheroplasts are nascently adsorbable, whereas most particles liberated from intact cells are adsorbable.

Bacteriophage particles in stocks of certain strains of T4 and T6 are incapable of adsorbing to host bacteria unless they have been activated by an adsorption cofactor (2, 3, 4). L-Tryptophan was found to be the most effective cofactor, being capable of inducing nearly maximal adsorbability at concentrations of 2×10^{-6} g/ml. The activation of T4 by cofactor is reversible; after the cofactor is diluted to nonactivating concentrations, 99% of tryptophan-induced adsorbability is lost in 3 to 5 min.

It has been reported (6, 12, 15, 16) that the effect of the cofactor is to interact with the T4 particle in such a way as to induce a configurational change in its tail fiber apparatus. In the absence of cofactor, cofactor-requiring T4 particles are often observed by means of electron microscopy to have most of their six tail fibers lying in close association with the sheath and collar regions of the phage structure. The same T4 observed in the presence of activating concentrations of tryptophan have most of their tail fibers in an extended state, free of the sheath and available for interaction with the host-cell receptors. It is thought that these changes in tail fiber arrangement can account for the observed change in the rate of sedimentation from $S_{20,w}$ = 1,100 for inactive particles that are streamlined with their tail fibers wrapped around the tail to $S_{20,w}$ = 850 for tryptophan-activated particles whose extended tail fibers exert a frictional drag as they move through the medium. Cummings et al. (7) suggested that alterations in the size of the phage head may also contribute to changes in the rates of sedimentation.

Gamow and Kozloff (12) postulated that the function of tryptophan in activating cofactor-requiring phage is to combine with a folic acid-like residue located in the baseplate of the T4 particle. This interaction results in a configurational change in a postulated hinge structure located in the baseplate, thus changing the orientation of the phage tail fibers.

Even though cofactor-requiring T4 particles were found to be incapable of forming plaques when plated directly with *Escherichia coli* strain B on cofactor-free agar, it was noted (3) that host bacteria that had previously been infected with cofactor-requiring T4 could form plaques on

[1] Presented in part by Dennis T. Brown in partial fulfillment of the requirements for a Ph.D. degree at the University of Pennsylvania.

[2] Present address: Department of Biological Sciences, Dartmouth College, Hanover, N.H. 03775.

bacteria growing on such media. Since the production of a plaque involves many rounds of infection, reproduction, and liberation of phage, it was thought that the daughter particles must somehow have been infectious. However, when the progeny phage produced in such plaques were isolated and tested by plating in the presence and in the absence of cofactor, they were found to require cofactor for adsorption just as the parent particle had. Anderson (3) attributed this rather surprising result to the possibility that the progeny phage had obtained cofactor from the host cells growing on synthetic agar.

In 1952, Wollman and Stent (25) named this state of adsorbability possessed by the newly released T4 "nascent activity" and termed phage which possessed this activity "nascent" phage as opposed to "quiescent" or cofactor-requiring phage. They demonstrated that nascent adsorbability was due to an active state which was lost much more slowly on dilution than tryptophan-induced adsorbability and suggested that nascent activity might be due to a tryptophan-like cofactor (perhaps tryptophan itself) binding to a special nascent surface possessed by the newly released T4 particles. This nascent surface was presumed to bind cofactor more firmly than the cofactor sites on quiescent phage, thus accounting for the slow loss of nascent adsorbability. The special surface was supposed to be lost more slowly than the tryptophan-like cofactor and was presumed not to be lost at all so long as cofactor was bound to it.

In 1956, Jerne (14) discovered that the adsorbability of tryptophan-activated T4 could be stabilized by "A8 serum." This was serum that had been collected from a horse only 8 days after the animal had received an injection of T4. Although this early serum had not developed an appreciable titer of neutralizing antibodies, it possessed the remarkable property in the presence of tryptophan of rendering T4 adsorbable to its host after the tryptophan had been removed. Jerne's results opened up another possibility: nascent adsorbability might be due to a substance acquired during its latent period that stabilizes the phage in its adsorbable state.

The results reported in this study indicate that the latter possibility is correct in a sense. Nascently adsorbable T4 has a lower density in CsCl than do quiescent particles, which suggests that the substance has a density that is lower than that of quiescent particles and a volume that is relatively large. When viewed by means of electron microscopy, quiescent particles appear "clean" with their long tail fibers wrapped around their sheaths, whereas nascently adsorbable particles are observed to have particles of "debris" attached to their baseplate regions with most of their long tail fibers free. A number of observations indicate that the material that is responsible for nascent adsorbability is derived from fragments of the host's cell wall. (i) Nascent adsorbability is destroyed by lysozyme. (ii) The adsorbability of tryptophan-activated T4 can be partially stabilized by a cell wall extract from *E. coli*. (iii) T4 particles that have been freshly liberated from host cells whose cell walls have been labeled with ^{14}C-labeled glucosamine contain this label but lose it as they spontaneously lose adsorbability. (iv) Whereas most of the particles liberated from whole cells have nascent adsorbability, very few of those liberated from spheroplasts whose cell walls have been largely destroyed by lysozyme before lysis possess this trait.

MATERIALS AND METHODS

Bacteria and bacteriophage. *E. coli* B has been described (2). The bacteriophage T4 used throughout this study was obtained by isolating a single clear (r^-) plaque from Anderson's stock labeled T4 5/10/45 (2).

General methods. The methods used were mainly those described by Adams (1). F medium contained per liter of distilled water: NH_4Cl, 1 g; KH_2PO_4, 1.5 g; Na_2HPO_4, 3.5 g; sodium lactate, 10.0 ml of a 60% syrup (Sigma Chemical Co., St. Louis, Mo.); and $MgSO_4$, 0.01 g (added separately). N broth contained per liter of distilled water: Bactotryptone, 10 g; yeast extract, 5 g; NaCl, 10 g; and glucose, 1 g. The *p*H was adjusted to 7.0 to 7.2 with 1 N NaOH. Agar was added to these liquid media at final concentrations of 1.0 and 0.6%, respectively, for plates and overlay. To prepare high titer stocks, 3 XD medium was used as described by Fraser (10).

Assays were made on N-agar to determine the total titer of a suspension of cofactor-requiring T4. Assays on F-agar were used to count the particles that were capable of adsorbing to bacteria in the absence of cofactor. The fraction (R) of the phage population capable of adsorbing in the absence of cofactor is expressed as the ratio of the number (F) of plaques obtained on F-agar to the number (N) of plaques obtained on N-agar.

Preparation of E. coli extracts. Crude *E. coli* extracts were prepared from 64 F hard-agar plates that had been seeded with *E. coli* B and incubated overnight at 37 C. The resulting bacterial lawn was harvested into 160 ml of F medium. The cell suspension was then pelleted in a clinical centrifuge at about 5,000 $\times g$ for 15 min and resuspended in a 10-ml volume of F medium. This very thick suspension was then treated three or four times in a French pressure cell (American Instrument Co., Inc., Silver Spring, Md.) at 25,000 psi, being careful to keep the temperatures of both the cell and the treated material below 4 C. The resulting paste was then treated with a droplet of chloroform to kill any surviving bacteria. These chloroform-treated cell wall fragments had little ability to kill T4 particles added later. They were quickly frozen at −78 C and stored at −20 C for later use.

Electron microscopy. The methods used were generally those of Anderson (5). Preparations were negatively stained with sodium silicotungstate at *p*H 7 to increase contrast and resolution. All preparations were studied by using a Siemens Elmiskop I electron microscope.

RESULTS

Preparation of nascently adsorbable T4. Wollman and Stent (25) demonstrated that their phage T4-38 exhibited a nascent state of infectivity by assaying the yield of phage produced in a one-step growth experiment and comparing the titers obtained on F and N agar. We have repeated this experiment with results that agree with those of Wollman and Stent. During the first 25 min of the latent period before the infected cells begin to lyse, the assays on F and N agar are identical, for each infected bacterium forms a plaque with equal efficiency on the two media. After 25 min, however, when the cells begin to lyse both titers increase, but the titers on F agar begin to fall below those on N by ever-increasing amounts; the freshly liberated particles slowly lose the ability to form plaques on F agar until after 2 hr only 1 particle in 1,000 forms a plaque on F. If such lysates are allowed to stand at room temperature for a day, the value of R (= Assay on F agar/Assay on N agar) falls to as low as 5×10^{-6}.

To study effectively the problem of nascent adsorbability, it was necessary to have readily at hand a stock suspension of phage known to be nascently active. This was accomplished by cooling to 0 C a suspension of infected bacteria in F medium after only 30 min of aeration. Chloroform was then added to induce premature lysis. A trace of deoxyribonuclease was also added to decompose the liberated deoxyribonucleic acid (DNA) and so reduce the viscosity. After standing at 0 C for 8 to 10 min, the titers on F and N gave R values between 0.6 and 1.0. It was found that the nascent adsorbability of the phage in such lysates could be preserved indefinitely by adding glycerol to a final concentration of 5% and quick-freezing small samples in an acetone–dry-ice bath at −78 C. After storage at −40 C for months, these lysates showed no measurable decrease in R, although some loss in total titer was observed. Therefore, these preparations provided a readily available source of nascently adsorbable phage for the studies that followed.

Factors affecting the rate of loss of nascent adsorbability. The initial observation that the nascent state of adsorbability is lost more slowly than tryptophan-induced adsorbability prompted a study of conditions which might be expected to change this observed rate. A number of factors had no observable effect. The rate was essentially constant between *p*H 6 and 9 and in NaCl concentrations between 4 M and 0 (distilled water); nor did the presence or absence of oxygen or of visible light affect the rate.

The experiment of Fig. 1 shows that tryptophan (and the other compounds and known cofactors such as phenylalanine and tyrosine in N) have no effect on the loss of nascent adsorbability; nor does the reactivation by tryptophan of a phage preparation which has lost part of its nascent adsorbability affect the rate of loss of adsorbability. The reactivated phage return to a state of adsorbability equal to that of the control and quickly lose their tryptophan-induced adsorbability when the tryptophan is removed. These

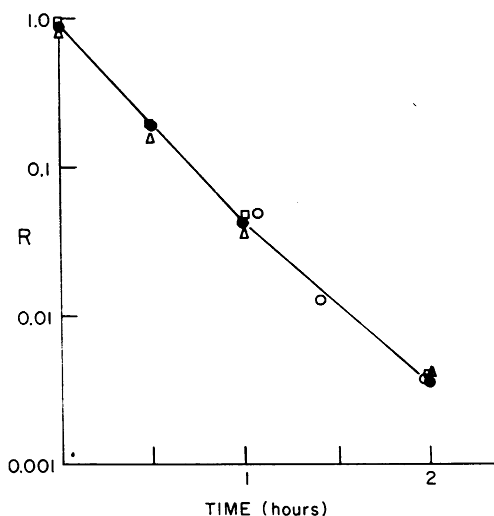
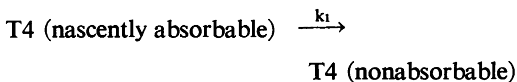


FIG. 1. *Effect of tryptophan and N medium on the rate of loss of nascent adsorbability of T4. Samples (0.1 ml) of a freshly thawed nascent phage preparation were added to each of four tubes containing: tube 1, 10 ml of F medium (●); tube 2, 10 ml of F medium containing 100 μg of L tryptophan/ml (△); tube 3, 10 ml of N broth (□); and tube 4, 10 ml of F medium (○). All samples were incubated at 37 C. At various times, samples of tubes 1, 2, and 3 were diluted and plated with B on F and N. The three samples lost nascent adsorbability at essentially identical rates, indicating that neither tryptophan alone nor the other components in N medium affect the rate of loss of nascent adsorbability. The sample in tube 4 was allowed partially to deactivate for 1 hr, at which time tryptophan was added to make the final concentration 100 μg/ml. After 3 min, the sample was diluted to a nonactivating concentration of tryptophan (0.01 μg/ml). After an additional 5 min to allow the phage to lose its tryptophan-induced activation, the diluted preparation was assayed on F and N. Here again, the R values are identical to those in the other three tubes.*

results disagree with those of Wollman and Stent (25) and will be discussed later.

Temperature did affect the rate of loss of nascent adsorbability as shown in the experiment of Fig. 2 in which the ratio R is plotted against the time of incubation at different temperatures. These results are in good agreement with the data reported by Wollman and Stent (25) and approach first-order kinetics for the initial period as though the reaction



$$\text{T4 (nascently absorbable)} \xrightarrow{k_1} \text{T4 (nonabsorbable)}$$

were the principal one occurring in this system. Plotting log k_1 against $1/T$, one can estimate the activation energy (E_a) for the reaction to be 11,000 cal.

The kinetic observations indicate a low temperature dependence for the conversion of the majority of the nascent phage to cofactor-requiring phage. On the other hand, a lack of homogeneity in the population could give an apparent value of E_a that is lower than that for any of the particles. In fact, at all the temperatures studied the rate of deactivation decreases as R approaches its minimal value of 5×10^{-6} to 5×10^{-5}. Some, but not all, of the particles that lose activation very slowly prove to be mutants that have reduced requirements for cofactor (4).

It is important to emphasize that nascent phage deactivate much more slowly than tryptophan-activated phage at each of the temperatures studied.

Electron microscopy of nascently adsorbable phage. The electron microscope was used to determine whether nascently adsorbable T4 possess any gross morphological differences from cofactor-requiring phage. In the following experiment, nascent phage were obtained by allowing infected bacteria to lyse on a carbon-coated electron microscope grid. *E. coli* B growing in the log phase in F medium were infected with tryptophan-activated T4*r* [multiplicity of infection (MOI) = 5]. After 5 min had been allowed for adsorption, the infected bacteria were pelleted. The supernatant fluid containing unadsorbed phage plus the cofactor was discarded and the cells were resuspended in fresh F medium. The resuspended cells were incubated for 20 min at 37 C, at which time a small droplet of the suspension was transferred to a prepared copper grid. The grid, held with forceps, was incubated for an additional 10 min at 37 C, after which it was washed with F medium and then negatively stained and placed in the microscope to dry.

Microscopic fields produced by this method were found to contain many unlysed bacteria as well as bacterial debris; however, some areas such as that shown in Fig. 3 were relatively clean. In this instance one can see the end of a bacterium which has broken open to release its contained phage onto the grid surface. One is struck by the fact that most of the phage have all six of their tail fibers in the extended state. On two of the particles the six tail fibers are pointed out with arrows, but many of the other phage may also have all six fibers extended. The release of all six tail fibers is generally not seen in purified cofactor-requiring phage even in the presence of high concentrations of tryptophan (6, 15).

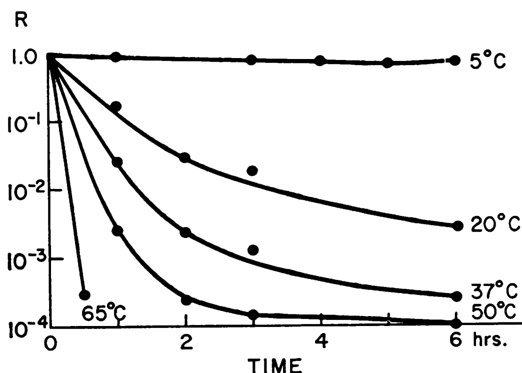


FIG. 2. *The deactivation of nascently adsorbable T4r⁻ at different temperatures. Samples (1 ml) of a freshly thawed nascent phage suspension were added to 9-ml volumes of F medium at the indicated temperatures and were assayed from time to time on F and N agar to determine the R value of the population, which is shown plotted against time.*

Comparison of the adsorption rates of nascently adsorbable T4 and of tryptophan-activated T4. If the rate of adsorption of a population of phage is limited by the rate at which the tail fibers can be made available for interaction with the host receptors, the nascently active phage, having all tail fibers extended, might be expected to exhibit a more rapid adsorption rate than cofactor-activated phage.

The rate of adsorption of cofactor-requiring phage activated by N broth, where its rate is a maximum (24), was compared to that of nascently adsorbable phage in F medium at 15 and 37 C. Contrary to expectation, the results (Fig. 4), indicate that nascent T4 in F adsorbs to B somewhat more slowly than does cofactor-activated T4 in N medium.

Data from five such adsorption experiments were averaged to give the rate constants recorded in Table 1. Adsorption rates were appreciable for both forms of T4 at both 37 and 15 C. This result

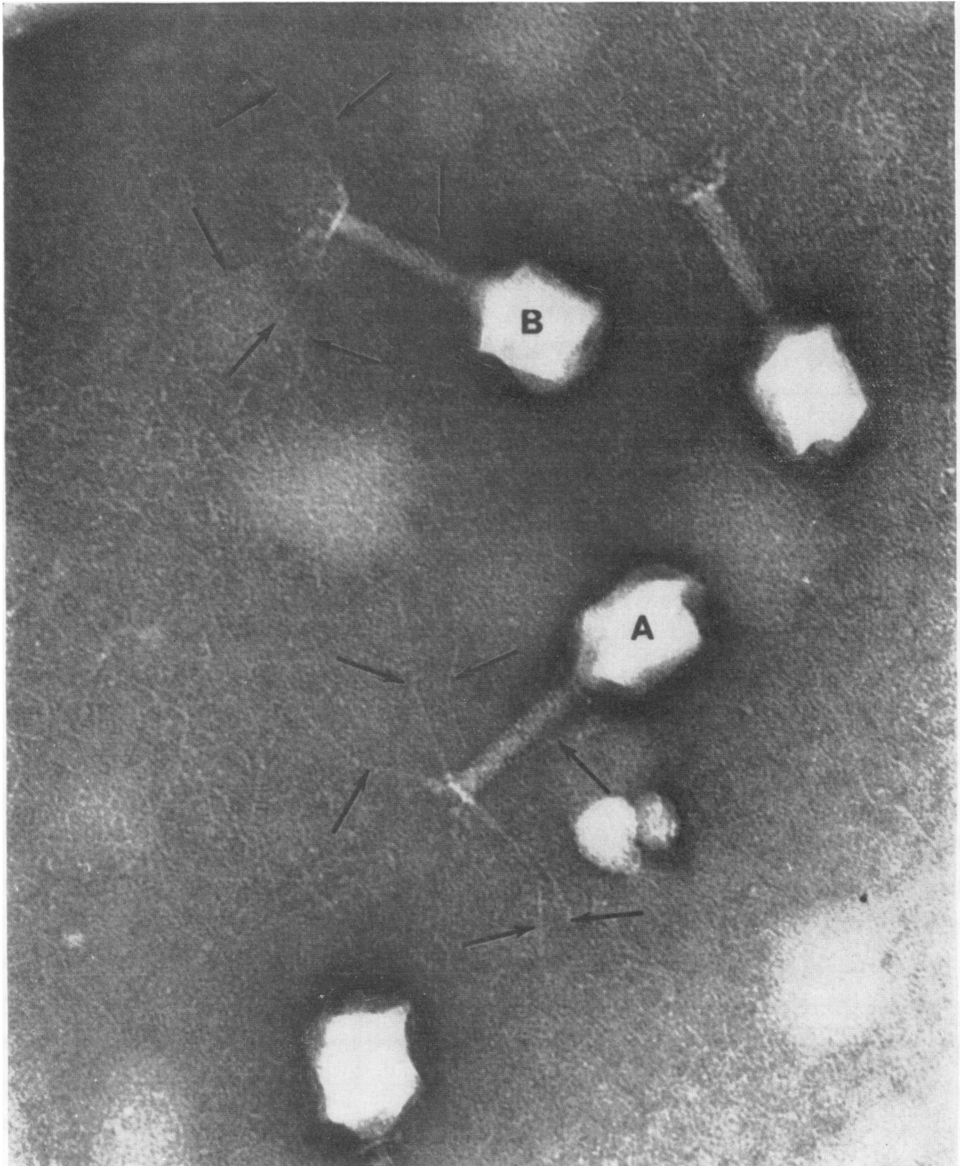


FIG. 3. *Electron micrograph showing nascent phage produced by allowing bacteria infected with cofactor-requiring T4 to lyse on an electron microscope grid maintained at 37 C. Tail fibers on two particles are pointed out with arrows. Stained with silico-tungstate.*

differs from the observations of Wollman and Stent (25), who reported that nascent phage adsorbed very slowly with a rate only 0.015 that of the same phage in broth at 15 C. It should be noted that the nascently adsorbable phage in F medium appear to adsorb at only a slightly slower rate than do cofactor-requiring phage in broth. On the one hand, this difference in rates may be due to some effect other than available cofactor; for instance, the different nutrients may have different effects on the bacterial surfaces. On the other hand, the lower apparent adsorption rate for the nascent phage may be due to its progressive loss of adsorbability during the experiment, whereas the cofactor-requiring phage in broth have sufficient available cofactor to en-

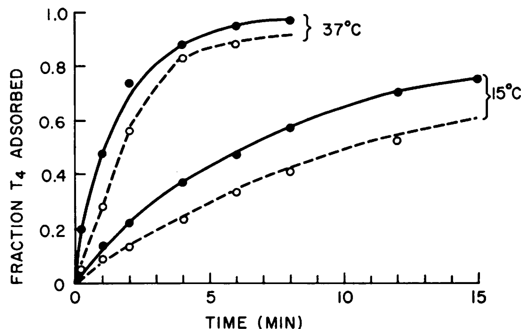


FIG. 4. *Comparison of the rates of adsorption of nascent T4r⁻ and of N broth-activated cofactor-requiring T4r⁻ at 15 and 37 C. Cofactor-requiring T4r⁻ was diluted into N broth. Nascent T4r⁻ from a frozen preparation was diluted into F medium. At time zero, a volume of E. coli B was added to each tube to make their final concentration 2 × 10^8 cells/ml and that of the phage 5 × 10^7 particles/ml. The reaction mixtures were incubated at either 37 or 15 C. At various times, samples of the reaction mixture were diluted 1/100 into medium containing anti-T4 serum at a concentration sufficient to inactivate 99.9% of the unadsorbed phage in 2 min. The infected bacteria were then plated on N agar. Solid line indicates T4 activated with nutrient broth; broken line indicates nascently active T4.*

TABLE 1. *Velocity constants for the adsorption of nascent T4 and cofactor-activated T4 at 15 and 37 C*[a]

Temp	Cofactor-requiring phage in broth	Nascently-active phage in F medium
15 C	(2.3 ± 0.5) × 10^{-10}	(1.9 ± 0.9) × 10^{-10}
37 C	(12.5 ± 0.3) × 10^{-10}	(8.2 ± 0.5) × 10^{-10}

[a] Values are expressed as milliliters per minute.

sure that they will all be adsorbable throughout the experiment. It was concluded from these experiments that nascently adsorbable phage possess an ability to adsorb to bacteria that is nearly equivalent to that of cofactor-activated phage.

Retention of nascent adsorbability by intracellular phage. The experiments described above have been confined to free phage that had been released from the host cell at the end of the normal 30-min latent period. The experiment of Fig. 5 was carried out to determine whether the nascent state of adsorbability can be retained by the phage inside the host cell beyond the normal latent period. If the nascent adsorbability were due to some special nascent cofactor which was incapable of diffusing out of the cell, one might expect the phage to retain their state of adsorbability throughout an extended latent period. If,

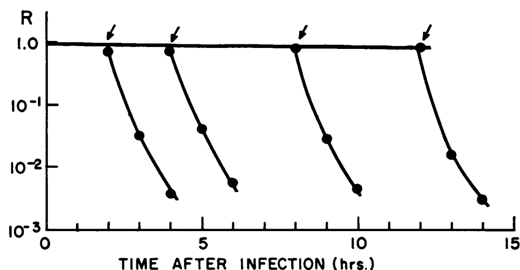


FIG. 5. *Effect of the extended latent period on the nascent state of adsorbability. E. coli B, rapidly growing in F medium, were infected with cofactor-activated T4r⁻, centrifuged, and resuspended as previously described. After incubation at 37 C for 26 min, KCN was added to the mixture to a final concentration of 6 × 10^{-3} M to prevent spontaneous lysis. The suspension of infected cells was stored at 37 C. At various time intervals, samples of this suspension were lysed with chloroform at 0 C and were assayed on F and N agar. The assay dilution tubes were then returned to 37 C and replated from time to time to follow the course of the phage activity after the delayed lysis. The top line shows the R value of the phage at the time of induced lysis (indicated by arrows). The descending lines represent the decrease in R with time at 37 C after the induced lysis.*

on the other hand, nascent adsorbability were due to a diffusable cofactor or to some time-labile configurational difference at the cofactor sites, one would expect the phage to lose its nascent adsorbability and be released from the cell in a reduced state of adsorbability.

The results of such an experiment are shown in Fig. 5, in which R is plotted against time after infection. It is shown that phage released from the host cell up to 12 hr after the normal latent period possess nascent adsorbability and that this adsorbability is lost only after the phage is released from the host cell. The fact that phage retained the nascent state of adsorbability so long as they remained inside the host cell was interpreted to support the notion that nascent adsorbability is due to some special "nascent cofactor" that cannot diffuse from the cell.

Nascent cofactor from bacterial cells. An attempt to find the nascent cofactor in extracts of uninfected *E. coli* B that had been grown in F is described in Fig. 6. Evidently, something similar to the nascent cofactor can be obtained from the uninfected *E. coli* cell. The activating ability of this nascent cofactor is considered to be rather low, for 6 hr was required to reactivate the phage from R = 10^{-5} to R = 10^{-3}, whereas the same phage had been released with R = 1.0 at the end of a normal 30-min latent period.

A situation similar to this was found by Jerne

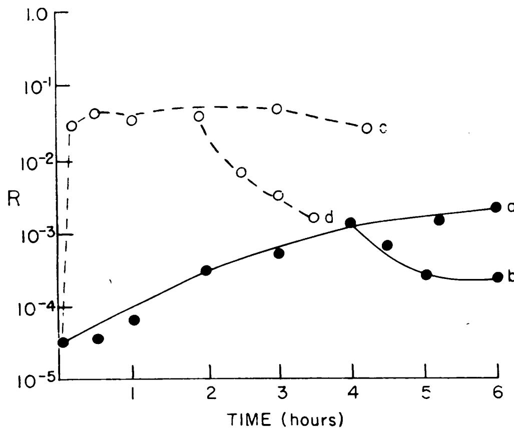


FIG. 6. *Reactivation of quiescent T4r⁻ by F-grown E. coli extract. T4r⁻ (R = 5 × 10⁻⁵) was added to 1-ml samples of E. coli homogenate that had been prepared as described in Materials and Methods. The resulting mixture was incubated at 37 C, and samples were diluted from time to time and plated on F and N with the result shown in curve a. Only a slight activation is produced by the extract in a 6-hr period. One of the dilution tubes taken from this reaction mixture was allowed to stand at 37 C and samples were replated at various intervals on F and N. The induced activity is lost at a rate (curve b) that is much slower than would have been expected for tryptophan-induced activity. Curve c shows the results of a similar experiment in which activation by E. coli extract occurred in the presence of 50 µg of L-tryptophan per ml and in which samples were diluted 1/1,000 at various times into cofactor-free F medium. They were assayed on F and N after incubation for 5 min had been allowed at 37 C to eliminate any tryptophan-induced activation. The 1/1,000 dilution tube taken at 2 hr was incubated further at 37 C and assayed from time to time on F and N agars to give the results plotted in curve d. L-Tryptophan greatly increases the rate of activation by the E. coli extract and the activated phage loses its adsorbability at the slow rate that is characteristic of nascently adsorbable phage.*

(14) in his studies of a phage-specific antiserum produced during the early period of immunization of a horse against T4. This antibody was found to activate T4 permanently, but at a rate that was very slow unless the phage had been activated by tryptophan. We have found (Fig. 6, curve c) that tryptophan similarly enhances the rate of activation of T4 by *E. coli* extracts and that, when the tryptophan is diluted out, this adsorbability is lost slowly at a rate equivalent to that of normally produced nascent phage (Fig. 6, curve d). It is also shown that the activated phage seem to retain this activity so long as they remain in the *E. coli* paste but the maximal observed activation represents only about 5% of the total phage population. This could well reflect competition between the activation reaction and the spontaneous loss of renascent adsorbability. No appreciable loss of phage titer was observed during this experiment, which is possibly a result of pretreating the extracts with chloroform.

Attempts were made to increase the activating properties of the *E. coli* extract by use of various purification procedures. The extract was centrifuged at 5 C for 10 min at about 6,000 × *g* in a clinical centrifuge. When the supernatant fluid and the resuspended pellet were tested for activating ability in the presence of 50 µg of tryptophan per ml, the supernatant fluid was found to possess no detectable activating material, whereas the pellet was found to induce adsorbability with about the same efficiency as did the original material. Attempts to remove the activating material from the whole paste by exhaustive dialysis at 5 C against F medium failed.

The above results indicate that the nascent cofactor is associated with the larger faster-sedimenting portions of the bacterial paste, possibly fragments of the cell envelope.

Since *E. coli* cell walls possess substrate sites for the enzyme lysozyme, this enzyme was tested for its effect on the activating properties of the *E. coli* extract. The resuspended pellet of a 10 min, 6,000 rev/min centrifugation of crude *E. coli* extract was treated with lysozyme at 3×10^{-5} g/ml and allowed to incubate at 37 C for 10 min. Cofactor-requiring T4*r*⁻ were then treated with this material for 50 min at 37 C in the presence of 50 µg of tryptophan per ml. A control containing pelleted material had undergone a similar incubation procedure, but it was not treated with lysozyme.

The results (Table 2) indicate that lysozyme destroys the activating ability of this material. Other enzymes were tested for their effect on the activating properties of this pellet fraction, with uniformly negative results.

Effect of enzymes on the deactivation rate of nascently adsorbable T4. The experiments already described indicate that nascent adsorbability is due to a special nascent cofactor. To further characterize the chemical nature of this cofactor, the deactivation kinetics of nascent phage were studied in the presence of various enzymes. A suspension of freshly thawed nascent T4*r*⁻ was treated with the enzyme to be studied and was incubated at 37 C. Samples of this reaction mixture and a control without enzyme were diluted at intervals and plated on F and N media. The enzymes studied included Pronase (5×10^{-4} g/ml), trypsin (2.5×10^{-3} g/ml), deoxyribonuclease (1.0×10^{-3} g/ml), ribonuclease (1.0 ×

10^{-3} g/ml), phospholipase C (8.0×10^{-4} g/ml), and lysozyme (at 2.1×10^{-4} and 8.8×10^{-3} g/ml).

Lysozyme alone was found to increase the rate of deactivation with a concentrational dependence that is shown in Fig. 7. Lysozyme's activity is probably not due to its high net-positive charge (isoelectric point = *p*H 11) causing it to react nonspecifically with the phage particles, for trypsin with essentially the same isoelectric point at *p*H 10.8 (18) had no effect. On the other hand, the rate of deactivation is not proportional to lysozyme concentration and was much slower than might have been expected for lysozyme acting on a readily available substrate. It may well be that the lysozyme-sensitive site is associated with some other type of macromolecule on the phage particle that limits its accessibility to the enzyme. At any rate, it would appear that the nascent cofactor contains material that is susceptible to lysozyme.

TABLE 2. *Effect of lysozyme on the activating properties of E. coli extract*

Material tested	R value produced[a]
T4r^- + 50 μg of tryptophan per ml (no extract)	5×10^{-5}
T4r^- + 50 μg of tryptophan per ml + extract	3×10^{-2}
T4r^- + 50 μg of tryptophan per ml + lysozyme-treated extract (3×10^{-5} g of lysozyme per ml, 50 min at 37 C)	5×10^{-5}

[a] Measured after dilution to nonactivating tryptophan concentration.

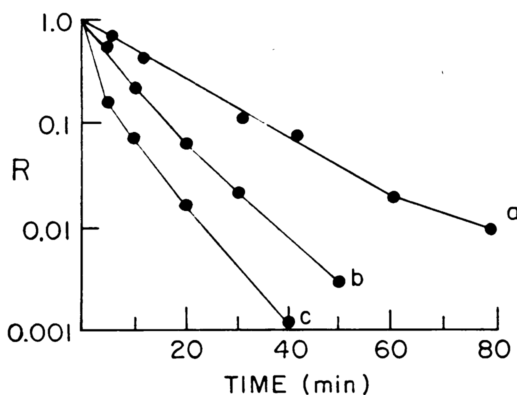


FIG. 7. *Effect of lysozyme on the rate of loss of nascent adsorbability. Freshly thawed preparation of nascent phage was allowed to incubate at 37 C alone (a) and in the presence of 2.1×10^{-4} g of lysozyme per ml (b) and 8.8×10^{-3} g of lysozyme per ml (c). At various times, the three preparations were assayed on F and N agar and the R value of each was plotted against incubation time.*

Buoyant density of nascently adsorbable T4. To determine how much of the relatively low-density cell wall material is bound to nascent T4, we have compared its density in CsCl with that of T4 from the same preparation that had been incubated at 37 C to acquire the need for a cofactor. The cofactor-requiring particles (R = 10^{-4}) band in peaks at ρ = 1.49 and ρ = 1.51 g/ml (Fig. 8). In contrast, the nascent particles (R =

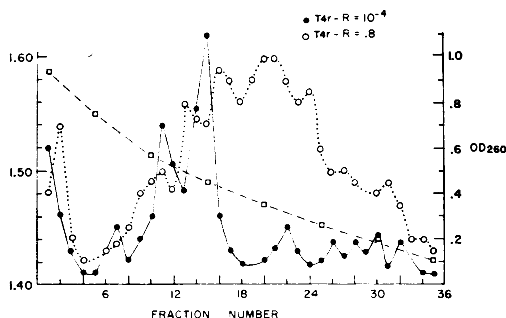


FIG. 8. *Densities in CsCl of nascently adsorbable T4r^- and of cofactor-requiring T4r^- from the same preparation. Nascent phage were prepared by the technique of one-step growth; 30 ml of E. coli B, rapidly growing in 3 XD medium at a concentration of 5×10^8 cells/ml, were infected (MOI = 5) with cofactor-requiring T4r^- in the presence of 10^{-4} g of added tryptophan per ml. After 5 min at 37 C had been allowed for adsorption, the infected bacteria were pelleted and resuspended in 10 ml of fresh 3 XD. The infected cells were incubated with aeration for an additional 26 min and then were quickly cooled in an ice-salt bath. Chloroform was added to lyse the cells and a trace of deoxyribonuclease was added to reduce the viscosity of the mixture. After 10 min at 0 C had been allowed to obtain maximal lysis, the mixture was divided into two 5-ml portions. One sample was allowed to remain at 0 C; the other was incubated at 37 C for 5 hr. The R value of the material stored at 0 C was then found to be close to 1.0, whereas that of the 37 C material was 10^{-4}. CsCl was dissolved in the lysates to a concentration of 0.715 g/ml. The samples were centrifuged in lusteroid tubes at 39,000 to 40,000 rev/min for 18 hr at 3 to 4 C (Spinco model L-4, SW 50 rotor). Fractions were collected dropwise and used to determine OD_{260} (optical density at 260 nm) profiles, viability profiles, and indices of refraction. If viability assays were to be done, the entire collection, dilution, and plating procedure (to addition of agar) was carried out at 5 C to reduce the loss of nascent adsorbability. The second sample was given a low-speed centrifugation to remove some of the fast-sedimenting debris. It was allowed to stand for 24 hr at 37 C to lose nascent adsorbability. Figure 10b shows the sedimentation profile of a 10-μliter portion of this sample.*

0.8) evidently have lower densities, for they distribute themselves over a broad region whose density ranges from 1.46 to 1.50 g/ml. Assays of the phage in various fractions indicated that the R value is about the same at all densities; apparently the activating nascent cofactor material comes in a variety of sizes.

Electron microscopy of nascent T4. If the nascent cofactor is capable of shifting the density of phage particles as much as we observe, it should be possible to see the attached material by electron microscopy. Freshly prepared lysates of nascent phage were found to contain too much bacterial debris to give unequivocal results. Therefore, freshly prepared lysates were purified by several rounds of alternate high- and low-speed centrifugation at low temperature. Approximately 40 to 60% of the phage population lost nascent adsorbability during this procedure. Figure 9 shows phage particles from several such preparations. Material can be seen attached to the baseplate regions of many particles. This material is seen to vary in amount from rather large pieces on some particles to small, hardly visible fragments on the tail pins of others. This observation agrees with the density-gradient studies in indicating that the material bound to the phage has a wide range of sizes. The arrow in Fig. 9a points out a piece of free material identical in size and appearance to that attached to the nearby phage particle. This fragment may be a piece of material which had been liberated from the tail of a phage particle which has lost its nascent adsorbability.

Binding of cell wall fragments to nascent phage. That nascent adsorbability is related to the interaction of phage with host cell walls was further demonstrated by the experiments presented in Fig. 10 and 11.

Since it is known that *N*-acetyl glucosamine is a primary constituent of the *E. coli* "rigid layer" (19, 20), *E. coli* B cells were grown in ^{14}C *N*-acetyl glucosamine to make their rigid layers radioactive. Nascent phage produced from infection of these hot cells were sedimented on sucrose gradients (Fig. 10) and banded on cell density gradients (Fig. 11) before and after losing nascent adsorbability.

As is shown in Fig. 10a, phage having nascent adsorbability are able to affect the sedimentation profile of ^{14}C-labeled fragments of the bacterial wall. Cofactor-requiring phage on the other hand do not (Fig. 10b).

When nascent phage prepared from ^{14}C-labeled cells are banded on CsCl (Fig. 11), the phage having nascent adsorbability are seen to band polydispersely in the gradient (*see* also Fig. 8). The ^{14}C is seen to band in regions corresponding to the infectivity peaks (Fig. 11a). It is also found that the ratio of counts ^{14}C to infectivity is higher for those phage taken from the less dense region of the gradient, indicating that phage banding in these regions have larger fragments of cell wall material bound to them. The cofactor-requiring phage ($R = 1 \times 10^{-4}$) in Fig. 11b are found to band in a more restricted region of the gradient and have less of the label associated with them than do the nascent phage in Fig. 11a.

Adsorbability of phage produced from spheroplasts. The preceding studies have indicated that nascent adsorbability is the result of the interaction of T4 phage with host cell wall material at some time during the latent period. If this is so, it should be possible to produce tryptophan-requiring phage directly from spheroplasts, cells which have had most of their walls removed enzymatically (11).

E. coli B cells were infected with T4r^- in the presence of tryptophan (MOI = 10). A sample of the cells was converted to spheroplasts in 16% sucrose by a modification of the method of Guthrie and Sinsheimer (13). After incubation for 30 min to allow development of daughter phage particles, the spheroplasts were diluted 1:100 at 0 C in F medium to rupture them and release the daughter particles. Only 16% of the phage produced by spheroplasts were able to form plaques on F medium, whereas 82% of the particles produced by control cells formed plaques on F medium (Table 3). The simplest explanation is that the removal of the cell wall prior to the release of progeny T4 prevents most daughter particles from becoming nascently adsorbable. The equivalence in burst size in experiment and control indicates that the presence of the cell wall itself is probably unimportant for phage production.

DISCUSSION

Particles of T4 are liberated from host cells in a state of nascent adsorbability in which they can readily adsorb to host cells without additional cofactor (25). Once liberated into the medium, however, the particles spontaneously lose this nascent adsorbability with a half-time of only 20 min at 37 C. Thus, particles in older stocks or in preparations that have been purified in the course of a few hours lose their adsorbability. They cannot adsorb to host cells unless they have been reactivated by a cofactor like L-tryptophan. Activation by tryptophan is reversible; it is so rapid that half-times of the forward and reverse reactions are measured in seconds.

Our results also indicate that nascent adsorbability is due to a substance we shall call "nascent cofactor" that is attached to the phage particle.

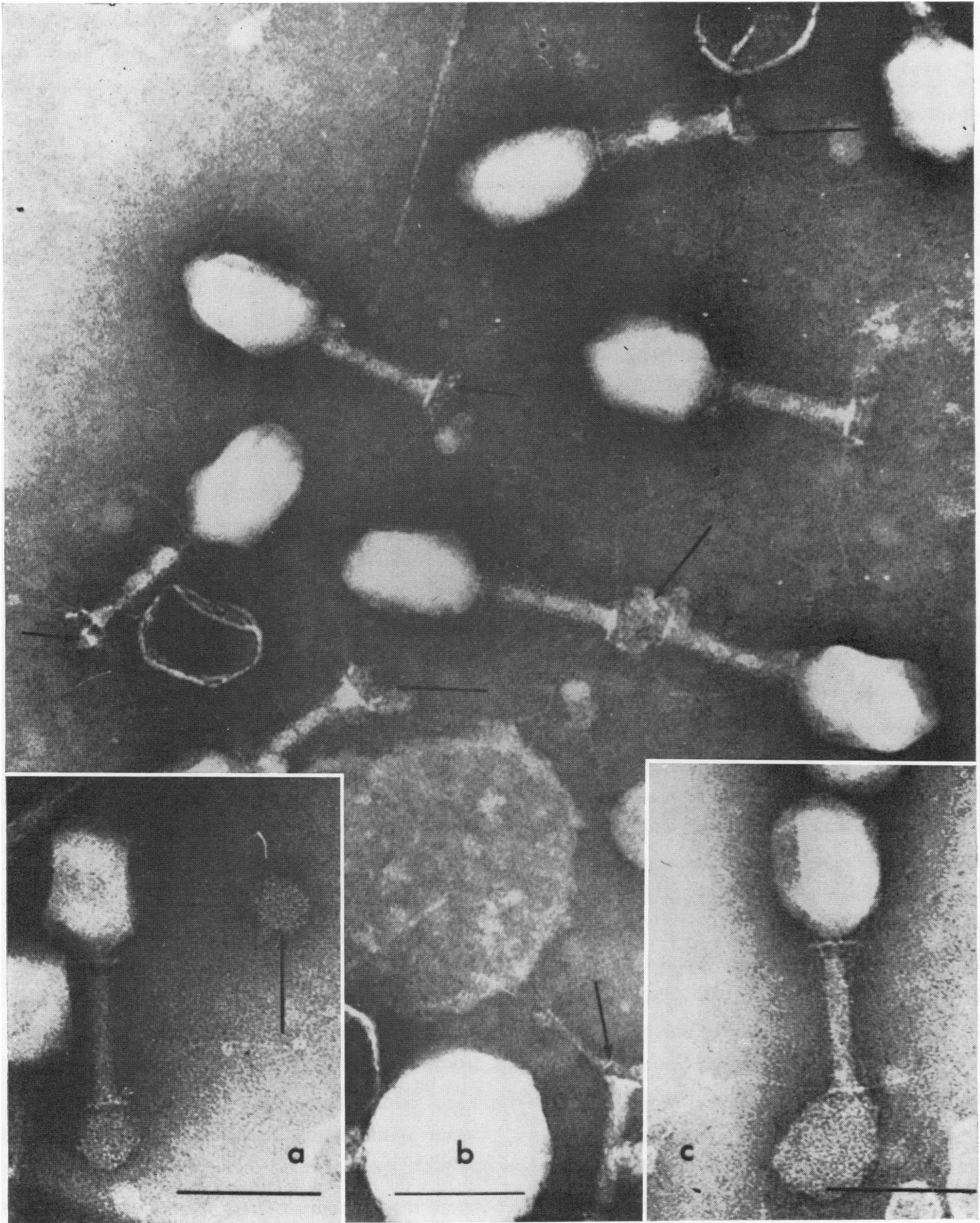


FIG. 9. *Electron micrographs of nascent T4. Phage were prepared by lysing infected bacteria at 28 min after infection by chloroform treatment. These nascently adsorbable particles were purified by several rounds of high- and low-speed centrifugation carried out at 4 to 5 C. The arrows point out material attached to the baseplate regions of the particles. In Fig. 9a, the arrow points out a fragment equal in size and shape to the one attached to the phage; this fragment may have been released by a particle which has lost adsorbability. Bars represent 100 nm.*

The nascent cofactor is evidently so large that it cannot diffuse from infected cells, for if their lysis is delayed in KCN, daughter phage particles can remain trapped inside for days without losing their nascent adsorbability. Furthermore, the

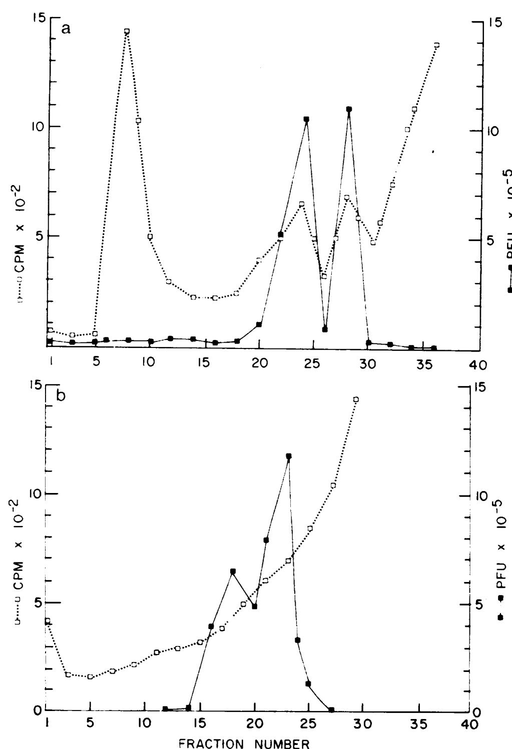


FIG. 10. *Sedimentation profile of nascent* (*a*) *and cofactor-requiring phage* (*b*) *prepared from cells which have their "rigid layers" labeled with* ^{14}C. *E. coli B were grown to a concentration of* 5×10^8 *cells/ml in F medium and then were diluted into 5 ml of F medium containing 0.05 mc of N-acetyl-*^{14}C*-D-glucosamine (specific activity, 10.4 mc/mM). The initial concentration of cells in the hot medium was* 10^7*/ml. After the cells had been allowed to grow to* 5×10^8*/ml, they were washed three times in cold medium. These cells were next infected with cofactor-requiring T4 at MOI = 10 in the presence of 50 μg of tryptophan per ml. The infected cells were centrifuged and resuspended in cofactor-free medium to a final concentration of* 2.5×10^9 *in 2 ml of F medium. This mixture was incubated at 37 C for 30 min in a micro-Fernbach flask. At the end of the incubation period, the mixture was cooled in an ice bath and treated with 0.2 ml of chloroform for 10 min. Two samples were collected. One 10-μliter sample containing nascently adsorbable T4 was layered quickly onto an ice-cold 5 to 30% sucrose gradient and spun at 20,000 rev/min for 10 min; equal-volume fractions were collected, assayed for infectivity, and counted for* ^{14}C *content. The results of this experiment are shown in Fig. 10a.*

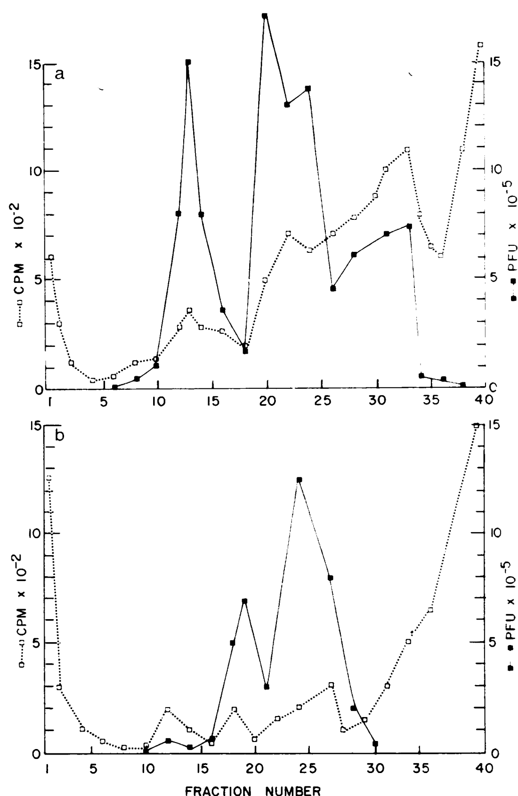


FIG. 11. *Density in CsCl of nascent T4* (*a*) *and of cofactor-requiring T4* (*b*) *produced from cells which have their rigid layers labeled with* ^{14}C. *Bacteriophage were prepared as described in Fig. 10. One sample was maintained at 0 C to prevent loss of adsorbability. A second was placed at 37 C for a period of 2 hr. CsCl was added to both while at 0 C to raise the density to about 1.475 g/cm*^{3}. *These samples were centrifuged in the SW 50-1 rotor at 35,000 rev/min for 18 hr at 2 C. Titer and counts/min are shown plotted against fraction number for nascent phage* (*a*) *and cofactor-requiring phage* (*b*).

mean buoyant density of nascently adsorbable T4 particles in CsCl is considerably lower than that of particles that have been allowed to lose their nascent adsorbability. This indicates not only that the nascent cofactor is large enough to affect the overall density of a T4 particle but also that the nascent cofactor has a density that is lower than that of particles that have been freed of nascent cofactor. The fact that nascent particles display a considerable range of densities indicates that the nascent cofactor can have a wide range of sizes. It is evident that this cofactor is not a discrete molecule but a substance or aggregation of substances. The electron microscope confirms this view: whereas particles in

TABLE 3. *Adsorbability of phage newly released from spheroplasts*

Expt	Total phage produced	R value
Control[a]	6×10^9	0.82
Experimental[b]	4×10^9	0.16

[a] Infected cells suspended in F medium + 16% sucrose.

[b] Infected cells suspended in F medium + 16% sucrose + 10^{-4} g of lysozyme/ml + 10^{-3} M ethylenediaminetetraacetate.

"purified preparations" that have lost their nascent adsorbability appear relatively "clean," nascently adsorbable T4 particles are seen to have pieces of material of various sizes adhering to their baseplate regions. This could well be the nascent cofactor.

By means of electron microscopy, it is also observed that T4 particles in purified preparations often have their long tail fibers wrapped around their tail sheaths, whereas nascent particles have all six long tail fibers freed from their sheaths, with their tips free to contact the surfaces of host cells and initiate the adsorption process (15, 22). L-Tryptophan also frees long tail fibers from the sheaths of quiescent particles to activate them, but oddly enough, even in a large excess of tryptophan, very few particles have all of their tail fibers visibly free of their sheaths (6, 15). Thus, it seems highly probable that this freeing of the tail fibers is the significant morphological configuration that enables T4 particles to be adsorbable, nascently or otherwise.

The rate of adsorption of nascent T4 particles is nearly equal to that of tryptophan-reactivated particles. One might suppose that the presence of what looks like bacterial wall debris associated with the baseplates of nascent particles would interfere with the complex function of this structure (22), but this material might readily merge with compatible substances endogenous to the bacterial surface when the baseplate region is brought into contact with the host cell wall. If so, this material would quickly be prevented from interfering with the function of the baseplate, and the rate of infection would scarcely be affected.

We have found that the cell wall fraction of *E. coli* can confer an active "renascent" state of adsorbability to many, but not to all, T4 particles that have lost their nascent adsorbability on standing. Two observations suggest that this wall material is very similar if not identical to the nascent cofactor. First, both the nascent adsorbability of the phage and the activating ability of the extracts are destroyed by lysozyme. Second, the renascent particles spontaneously lose their adsorbability at much the same rate as the nascent particles lose theirs.

Tryptophan greatly enhances the rate and extent to which the cell wall fraction activates T4, as though tryptophan prepared the phage for reaction with the wall fraction by extending its tail fibers. The wall material might then lock the particle in this adsorbable configuration in which it can remain even after the tryptophan has been removed. It seems probable that here tryptophan acts in much the same way as it does in promoting the permanent activation of T4 by A8 serum (14). It would appear that cofactors like tryptophan reversibly *activate* T4 particles by extending their long tail fibers, whereas substances like the nascent cofactor and A8 serum *stabilize* the particles in this adsorbable configuration.

The susceptibility of nascent adsorbability of T4 to egg-white lysozyme suggests that β-1-4 bonds between *N*-acetyl-glucosamine residues are essential to the activity of the nascent cofactor. These bonds probably are not readily accessible to the enzyme, however, for the rate of lysozyme action is not proportional to its concentration. Nascent phage produced from cells that had been prelabeled with *N*-acetyl-^{14}C glucosamine are found to exhibit a variety of densities that is paralleled by the density profile of this radioactive material. These phage also show a larger amount of label associated with the less-dense particles than with the phage of greater density.

Since the radioactive label in these experiments is incorporated into the rigid layer of the cell wall and since the loss of nascent adsorbability coincides with loss of label from newly released phage, it may be concluded that the nascent cofactor contains (in part at least) some of the host cell wall mucopolymer layer.

In light of the evidence presented here, it is possible to present a reasonable model for the nascent state of adsorbability. Since the nascent cofactor is an activity stabilizer rather than a phage activator, one can assume that this cofactor is bound to the phage during the time that the tail fibers are in the extended state.

Electron microscopy indicates that the cell wall material may be attached to the baseplate and tail-pin region of the phage structure. The baseplate of the phage may acquire cell envelope material as it is formed within the cell and sometime during the latent period either prior to the synthesis of tail fiber or before the tail fibers are retracted. On the other hand, the baseplate of the finished particle may associate with a portion of the cell wall of the infected bacteria. Since the

cell membrane is interposed between the cytoplasm and the cell wall proper, one must either include the membrane in the fragment bound to the phage or assume that the cell membrane has been broken down at this point, making possible direct access to the cell wall.

The location of the nascent cofactor on the underside of the baseplate may account for its ability to prevent the retraction of the tail fibers. Location of the hinge (12, 17) which allows the tail fibers to change orientation is not known but could well be in the area where the baseplate is attached to the tail fiber. The cell wall material bound to the underside of the baseplate may act in such a way as to prevent this hinge from working, thus creating a rigid tail fiber-extended configuration. This might be accomplished by linking the tail fiber to the tail pin. The loss of nascent adsorbability would be the result of removal of the cell debris from the baseplate, freeing the hinge and allowing the tail fibers to bind to the sheath. The forces removing the nascent cofactor from the phage may simply be due to kinetic forces acting on the rigid tail fibers, eventually freeing the hinge. The essential aspects of this model are depicted diagrammatically in Fig. 12. Particle A represents a T4 particle in the nascent state. The nascent cofactor is shown cross-linking the tail pins to the tail fibers, maintaining them in an extended state. T4 may lose nascent adsorbability in two ways. (i) The nascent cofactor may be removed from the baseplate region as in particle C, allowing the retraction of the tail fibers, or (ii) the tail fibers may free themselves from the cofactor and retract, leaving the cofactor attached to the baseplate as in particle B.

MODEL FOR NASCENT ACTIVITY

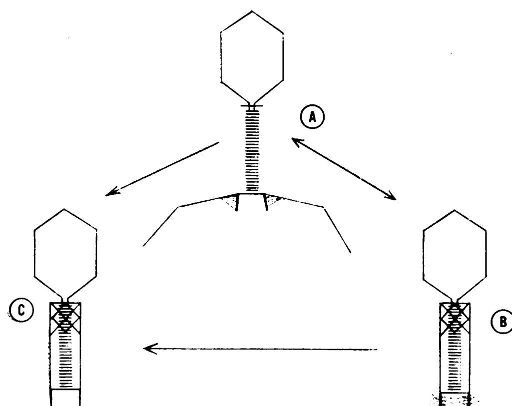
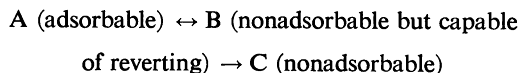


FIG. 12. *Model for nascent adsorbability.* (*See text for explanation.*)

The reaction A $\rightarrow$ B may be reversible, allowing the phage to return to the nascent state if the tail fibers are reassociated with the cofactor after displacement by some phage activator. [This may account for the return to the nascent state after treatment with tryptophan in the system of Wollman and Stent (25).] The reaction A $\rightarrow$ C would not be reversible, as the cofactor is gone from the baseplate. It would also be possible for particles in the B state to convert to the C state, losing their ability to revert back to the A state. Thus, an overall reaction might be



A (adsorbable) $\leftrightarrow$ B (nonadsorbable but capable of reverting) $\rightarrow$ C (nonadsorbable)

The reaction A $\leftrightarrow$ B $\rightarrow$ C may account for the shape of the curves obtained from the studies of the rate of loss of adsorbability of nascent phage. The deactivation curve (Fig. 2) of T4r^- seems to possess an initial region of linearity, followed by a decrease in deactivation rate prior to obtaining the lowest observed R value. The low R value observed for T4r^- was found to be $R = 5 \times 10^{-6}$. This change in rate may be the result of $k_{B \rightarrow C}$ being less than $k_{A \rightarrow B}$.

The reactivation of phage by the cell wall fraction of *E. coli* extracts is presumed to follow the model just described, with the exception that the tail fibers are first extended by tryptophan. If the nascent cofactor is made up of sections of the cell wall consisting of protoplasmic membrane and complete multilayered wall, increase in the deactivation rate by lysozyme may be by removal of some of the cell wall layers, leaving behind the membrane and other protein-containing layers. Under these circumstances, the rate of loss of nascent adsorbability may not reflect the digestion by lysozyme, as there would still be lysozyme-insensitive material to be removed.

The spontaneous loss of nascent adsorbability by free phage could be due to a simple dissociation of nascent cofactor from the phage. On the other hand, it is conceivable that spontaneous deactivation is due to the action of the same lytic enzymes in T4 that hydrolyze cell wall material when T4 is adsorbed to host cells (19, 21, 23). In the latter case, to account for the intracellular retention of nascent activity, one would have to assume that these enzymes are either blocked or cannot reach their substrates during an extended intracellular sojourn of the virion.

Three possible models may be suggested to explain how phage obtain these cell wall fragments. (i) Phage particles maturate with their baseplates in association with the cell envelope. (ii) At some time during the latent period, phage attach by their baseplates to the cell envelope.

(This attachment need not be permanent and at any given time only a few of the intracellular phage particles may actually be in contact with the wall structure.) (iii) Fragments of wall material might be obtained during the physical breakdown of the cell envelope (lysis).

The plausibility of the third model is strengthened by observations of T4-infected bacteria in

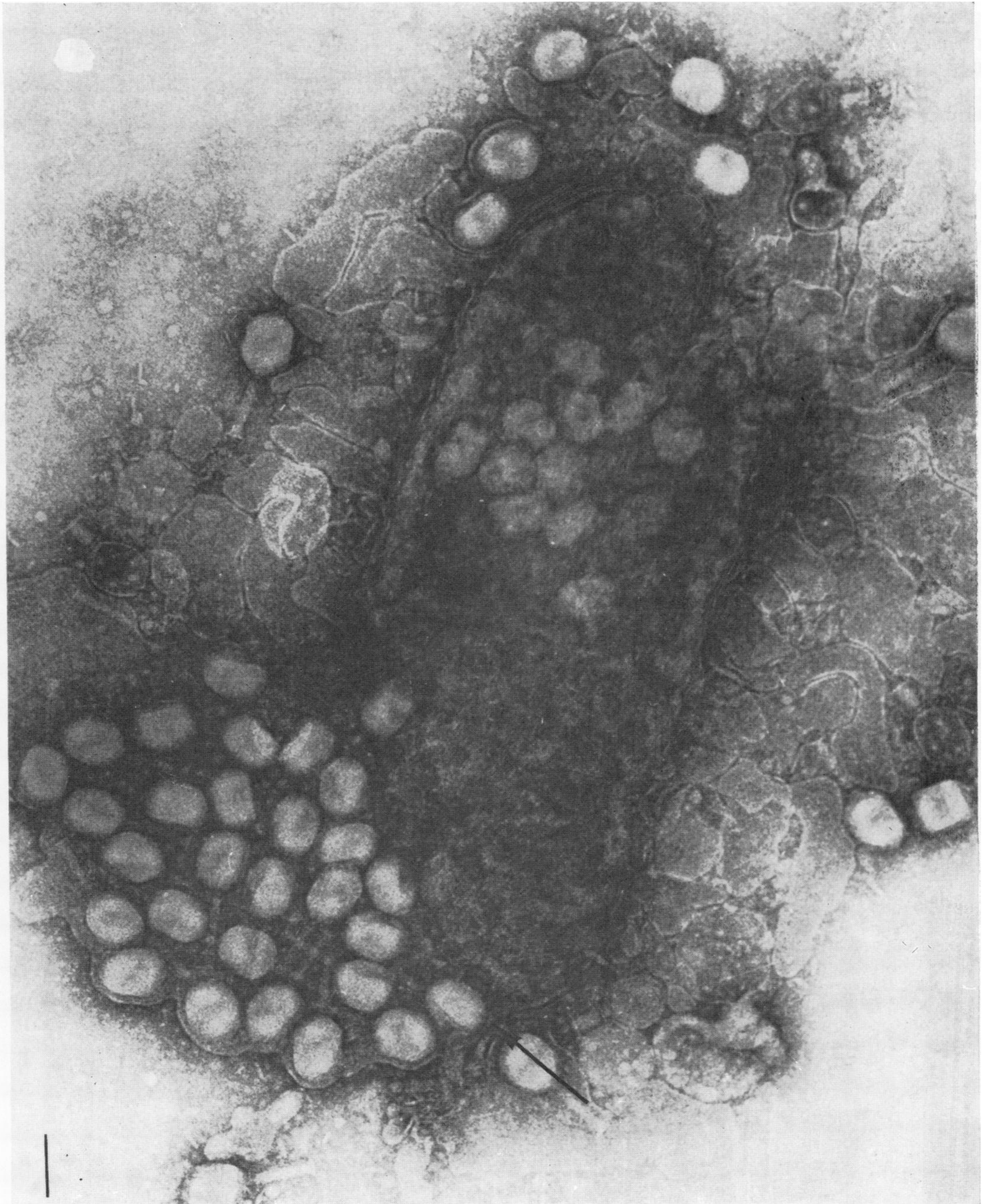


FIG. 13. *Electron micrograph of a bacterium infected with cofactor-requiring T4 in the process of lysis. The arrow points out a membrane-like structure which seems to surround the newly released particles. This membrane seems to be continuous with the bacterial cell wall.*

the process of lysis. By briefly exposing infected bacteria to chloroform vapor on an electron microscope grid, cells can be observed in the process of releasing their contained phage. In Fig. 13, the wall structure of a bacterium seems to be broken down in some areas where progeny phage are seen spilling out, although outside the normal confines of the host cell some of the progeny particles seem to be retained by a membrane-like structure which is continuous with the bacterial envelope. (This structure may consist of the lipid layers of the envelope.) It may be that the phage obtain the fragments of the rigid layer while retained inside this baglike structure during a temporary state of partial lysis. It thus seems possible that, much as certain animal viruses acquire their envelopes from nuclear (8) or cytoplasmic (9) membranes, the T4 phage particle acquires its nascent cofactor from cell wall material at the time of its release from the cell.

ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of John M. Mackenzie, Jr. Manfred Bayer contributed many helpful suggestions in the preparation of this manuscript.

This investigation was supported by Public Health Service training grant 5-TO1 GM-00694-07 to the University of Pennsylvania from the National Institute of General Medical Sciences, by Public Health Service grants FR-05539 and CA-06927 to the Institute for Cancer Research, by grant GB-4640 from the National Science Foundation, and by an award to Dartmouth College from the Brown-Hazen Fund administered by the Research Corporation of New York. An appropriation from the Commonwealth of Pennsylvania to the Institute for Cancer Research is gratefully acknowledged.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Anderson, T. F. 1945. The role of tryptophan in the adsorption of two bacterial viruses on their host *E. coli*. J. Cell. Comp. Physiol. **25**:17–26.
3. Anderson, T. F. 1948. The activation of the bacterial virus T4 by L-tryptophan. J. Bacteriol. **55**:637–649.
4. Anderson, T. F. 1948. The inheritance of requirements for adsorption cofactors in the bacterial virus T4. J. Bacteriol. **55**:651–658.
5. Anderson, T. F. 1962. Negative staining and its use in the study of viruses and their serological reactions, p. 251–262. *In* R. J. C. Harris, (ed.), The interpretation of ultrastructure, vol. 1. Symp. Int. Soc. Cell Biol. Academic Press, Inc., New York.
6. Cummings, D. J. 1964. Sedimentation and biological properties of T phages of *Escherichia coli*. Virology **23**:408–418.
7. Cummings, D. J., V. A. Chapman, and S. S. DeLong. 1969. The sedimentation and conformational variance among T-even bacteriophages. Virology **37**:94–108.
8. Darlington, R. W., and L. H. Moss, III. 1968. Herpes virus envelopment. J. Virol. **2**:48–55.
9. Davis, B. D., R. Dulbecco, H. N. Eisen, H. S. Ginsberg, and W. B. Wood, Jr. 1967. Microbiology. Harper and Row Publishers, New York.
10. Fraser, D., and E. A. Jerrel. 1953. The amino acid composition of T3 bacteriophage. J. Biol. Chem. **205**:291–295.
11. Fraser, P., H. R. Mahler, A. L. Shug, and C. A. J. Thomas. 1957. The infection of sub-cellular *E. coli* strain B with a DNA preparation from T2 bacteriophage. Proc. Nat. Acad. Sci. U.S.A. **43**:939–947.
12. Gamow, R. I., and L. M. Kozloff. 1968. Chemically induced cofactor requirement for bacteriophage T4D. J. Virol. **2**:480–487.
13. Guthrie, G. D., and R. L. Sinsheimer. 1963. Observations on the infection of bacterial protoplasts with the DNA of bacteriophage ϕX 174. Biochim. Biophys. Acta **72**:290–297.
14. Jerne, N. K. 1956. The presence in normal serum of specific antibody against bacteriophage T4 and its increase during the earliest stages of immunization. J. Immunol. **76**:209–216.
15. Kellenberger, E., A. Bolle, E. Boy de la Tour, R. H. Epstein, N. C. Franklin, N. K. Jerne, A. Reale-Scafate, J. Sechaud, I. Bendet, D. Goldstein, and M. A. Lauffer. 1965. Functions and properties related to the tail fibers of bacteriophage T4. Virology **26**:419–440.
16. Konner, L., and L. Kozloff. 1964. The reaction of indole and T2 bacteriophage. Biochemistry **3**:215–223.
17. Kozloff, L. M. 1968. The large DNA bacteriophages, p. 435–496. *In* H. Fraenkel-Conrat (ed.), Molecular basis of virology. Reinhold Book Corp., New York.
18. Long, C. 1961. Biochemistry handbook. D. van Nostrand Co., Inc., Princeton, N.J.
19. Martin, H. H. 1966. The biochemistry of bacterial cell walls, p. 457–484. *In* Paul D. Boyer (ed.) Annual review of biochemistry, vol. 35. Annual Reviews, Inc., Palo Alto, Calif.
20. Salton, M. R. J. 1964. The bacterial cell walls. American Elsevier Publishing Co., New York.
21. Sechaud, J., E. Kellenberger, and G. Streisinger. 1967. Permeability of cells infected with T4*r* and *r*+ phages. Virology **33**:398–404.
22. Simon, L. D., and T. F. Anderson. 1967. The infection of *Escherichia coli* by T2 and T4 bacteriophages as seen in the electron microscope. II. Structure and function of the baseplate. Virology **32**:298–305.
23. Weidel, W., and J. Primosigh. 1957. Die gemimsame Wurzel der Lyse von *Escherichia coli* durch Penicillin oder durch Phagen. Z. Naturforsch. **12b**:421–427.
24. Wollman, E. L., and G. S. Stent. 1950. Studies on the activation of T4 bacteriophage by cofactor. I. The degree of activity. Biochim. Biophys. Acta **6**:292–306.
25. Wollman, E. L., and G. S. Stent. 1952. Studies on the activation of T4 bacteriophage by cofactor. IV. Nascent activity. Biochim. Biophys. Acta **9**:538–550.