ABORTIVE INFECTION OF A STRAIN OF ESCHERICHIA COLI BY COLIPHAGE T2[1]

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During the course of experiments concerning the reproduction of bacteriophage T2r1 in starved *Escherichia coli*, strain K12, under conditions of low multiplicities of infection, it was observed that the total number of infective centers, that is, plaques formed by free phage and infected bacteria, was usually substantially lower than the original phage input. This has been reported since by Benzer (1952) to be true for T2r adsorbed on starved B at low multiplicities as well and has been called "abortive adsorption". We prefer to use the less committal term "abortive infection" since the data do not as yet permit us to isolate the specific phase of infection during which the loss of infectious centers occurs. Some aspects of this phenomenon, as well as some of the characteristics of adsorption and growth of T2r1 in strain K12, are reported below.

MATERIALS AND METHODS

T2r1 (referred to hereafter as T2), a rapid lysing variant of T2, obtained from Dr. N. Visconti, was used throughout these experiments. High titer stocks of the phage were obtained by harvesting from confluently lysed agar plates according to the method of Adams (1950). The concentrated phage was washed three times by high-speed centrifugations (21,000 G) in a Servall centrifuge for 60 to 90 minutes and resuspended in buffer after each centrifugation. Between high-speed centrifugations the suspension was centrifuged for 10 minutes at 2,500 rpm in an International size 1 centrifuge to remove large bacterial fragments and residual agar. The purified suspension then was passed through a Selas filter for sterilization.

Escherichia coli, strain K12, was used as the host throughout these experiments. Unless otherwise stated, the preparation of the cells was the following: A culture growing logarithmically in synthetic medium was centrifuged three times for 10 minutes at 2,500 rpm, resuspended in buffer after each centrifugation, and starved for three hours at 37 C under aeration. At the end of the starvation period, the cells were centrifuged, resuspended in buffer, and used.

Logarithmically growing *E. coli*, strain B, in nutrient broth was used as the indicator strain for free T2 and T2 infected K12, according to the agar layer method (Adams, 1950).

The synthetic medium M9 (Watson, 1950) was used throughout these experiments.

The buffer employed was made up of NaCl, 0.15 M; $MgSO_4$, 10^{-3} M; Na_2HPO_4, 0.01 M; and the pH was adjusted to 6.5 with HCl.

Nutrient broth (Difco), 0.8 per cent, containing 0.1 N KCl, was used as the nutrient broth liquid culture medium and nutrient adsorption medium.

Bacterial assays and determinations of bacteria surviving infection were performed by plating over a thin bottom layer in 40 ml of nutrient broth supplemented with 2 per cent agar without the addition of NaCl. This method has given consistently good results at the multiplicities of infection reported in this paper and affords a great saving of antiserum.

High titer anti-T2 serum was obtained by the method described by Adams (1950). The antiserum was diluted to a concentration which would inactivate 90 per cent of the free phage per minute and was left in contact with the phage-bacterial mixture for 5 minutes at 37 C. The antiserum action was terminated by immediate 10^3-fold dilution in iced buffer.

A Westinghouse WL-780-30 sterilamp at a

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distance of 12 cm was used as the ultraviolet light source in the irradiation studies.

Other pertinent methods will be described below in association with specific experiments.

RESULTS

The phenomenon of abortive infection. The data presented in figure 1 are typical of experiments in which the kinetics of phage adsorption and the fate of infected bacteria are followed as a function of time at 37 C. Cells, washed and starved as described above, were added to enough T2 to give a final multiplicity (ratio of adsorbed phage to total bacteria) of 0.18. Under these conditions, the frequency of multiple infection is about 1 per cent. To avoid multiple infection the multiplicity of infection was generally kept between 0.01 and 0.1 (lower than reported in this experiment). Aliquots were removed from the adsorption mixture at the times indicated and diluted in iced buffer to stop further adsorption. An aliquot of the adsorption mixture was centrifuged and the supernatant titrated for free phage. The total number of infectious centers was determined by plating an aliquot of the adsorption mixture.

The total infectious centers, free phage plus infected bacteria, thus obtained, minus the titer of free phage should be equal to the number of infected bacteria. Actually, as figure 1 indicates,

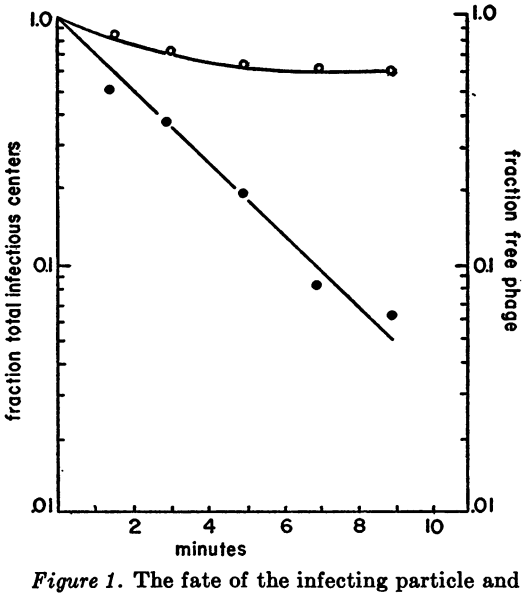

Figure 1. The fate of the infecting particle and the kinetics of phage adsorption. ○: fraction total infectious centers; ●: fraction free phage.

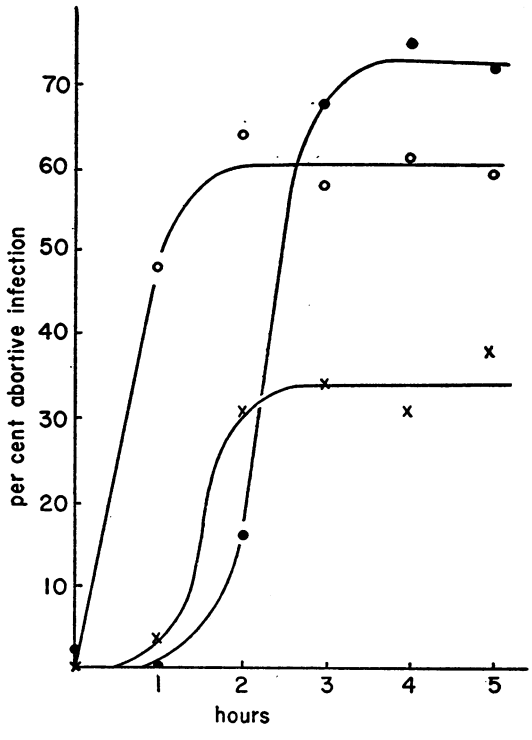

Figure 2. Abortive infection as a function of starvation time in buffer. All determinations were made by platings in triplicate.

the number of infected bacteria capable of plaque formation is approximately 60 per cent of the number of infected bacteria and does not vary appreciably with time in the adsorption mixture. The remainder are lost in terms of recoverability as plaques, or, in Benzer's terminology, are "abortively adsorbed".

The effect of starvation time in buffer. Figure 2 presents the results of experiments in which abortive infection was followed as a function of starvation time. Bacteria became susceptible to abortive infection as starvation progressed. The final frequency remained constant over a period of hours in any particular experiment although the time of onset and final frequency of abortive infection varied from experiment to experiment.

Starvation by limiting specific metabolites. Experiments were performed to see whether the starvation for either carbon or nitrogen would have varying effects on abortive infection. Cell growth was limited by reducing the NH_4Cl concentration in M9 to 0.0125 per cent and glucose to 0.05 per cent. These concentrations limit

TABLE 1

Abortive infection of cells whose growth had been limited by glucose, NH_4Cl, or adenine

HOURS AFTER REACHING MAXIMUM CONCENTRATION	PER CENT ABORTIVE INFECTION FOUND		
	Glucose limiting	NH_4Cl limiting	Adenine limiting
Log phase	0*	0	0
Log phase	0*	0	2
Log phase		10	
2	44*	67	
6	33*		36
9	68*		49
12	74*	65	53
12		47	42
13		34	
13		64	
18			63
18			21
20		58	
20		37	
20	50*	30	
20	51*	30	
24	44*		
24	46†		
29	13*		
41	64*		
41	19*		
43		43	
43		0	
45		41	
45		30	

Multiplicity of infection was kept at about 0.01, and adsorption of phage was allowed to proceed in the presence of about 10^9 bacteria.

These results were obtained by averaging the plaque counts of three plates each for free phage and total infectious centers taken from the adsorption tube at a time when more than 80 per cent of the virus was adsorbed onto the bacteria.

* Per cent determined by averaging results from complete adsorption curves as in figure 1.

† Average of four independent determinations from a single culture.

growth after the cells reach approximately the same density (5×10^8 per ml). To test whether the phenomenon was fairly general, an adenineless strain of K12 limited in growth on 5 μg adenine sulfate per ml also was tested.

Starvation time was measured from the point cultures reached maximum density on the limiting substrate to the time they were removed from the starvation medium by centrifugation. The data can be found in table 1.

Most of the cultures in which growth was limited by a limiting substrate demonstrated abortive infection. There is no evidence, again, of a continued increase in the amount of abortive infection as a function of time of starvation. There is also a wide variation of the extent of abortive infection from culture to culture, which seems also to be independent of the limiting substrate. The fact that one 43 hour culture starved for nitrogen showed no abortive infection seems to indicate that there is, at best, only little bacterial inactivation under these conditions. Repeated determinations of viable bacteria in these cultures were in close agreement with concentrations determined densitimetrically. If the major cause of abortive infection were due to adsorption on dead bacteria, differences between viable counts and density measurements should be significant. Ryan (1953) also has found that there is very little bacterial inactivation under conditions of glucose limited growth.

Some tests of techniques. Experiments testing the possibility of the inactivation of infected bacteria in iced buffer (Gross, 1954) showed no change in total infectious centers over periods up to two hours. Infected cells maintained in buffer at 37 C before plating are inactivated to some extent. However, the rate of inactivation during the first twenty minutes is so slow that it could have only a negligible effect during the adsorption period. When phage adsorption was carried out on glucose starved cells with and without washing, the extent of abortive infection was found to be the same. The dilution and washing techniques employed throughout these experiments can be disregarded therefore as causative factors for the phenomenon studied.

The reversibility of the starvation effect. Since actively growing cultures show no abortive infection, experiments were performed to test the reversibility of the phenomenon by incubating starved cells in the presence of growth media prior to adsorption. The experiments involved starving cells on limited glucose as indicated in table 2, and dividing the cultures into two parts after washing. One part was distributed into three adsorption tubes containing phage suspended in buffer, broth, and M9, respectively. The other half of the culture was suspended in either buffer broth, or M9; and phage was added after a half hour incubation at 37 C. In both cases the adsorption time was 5 minutes and dilutions were carried out in iced buffer.

TABLE 2

The effect of pretreatment of the host before infection

CULTURE	NO PRETREATMENT, PER CENT ABORTIVE INFECTION			0.5 HOUR PRETREATMENT, PER CENT ABORTIVE INFECTION		
	Buffer	Broth	M9	Buffer	Broth	M9
1	47	49	42	48	0	0
2	45	51	48	41	0	0
3	44	53	47	44	5	0
4	58	51	59	62	10	0
5	37	41	40	35	0	7
6	37	31	35	31	0	0
7	67	59	63	59	0	3
8	41	37	31	44	0	0

Escherichia coli, strain K12, starved on limiting glucose for 20 hours was used throughout these experiments.

Adsorption media were: buffer, 50 per cent broth, and 50 per cent M9.

In the case of no pretreatment, phage suspended in full strength buffer, broth, or M9 was added to cells suspended in buffer. Where pretreatment is indicated, starved cells were incubated for 30 minutes in full strength buffer, broth, or M9 before the addition of phage suspended in buffer.

All platings were done in triplicate.

Where the recovered plaques were equal to or slightly in excess of the input, the per cent abortive infection was noted as 0.

The data indicate a complete reversal of abortive infection when cells are allowed to remain in a medium supporting growth for thirty minutes before the addition of phage. The fact that broth added along with the phage at the outset of adsorption has no effect indicates that abortive infection is not due to the absence of "penetration" or "growth" factors. A few experiments done with various quantities of cell-free extracts of logarithmic phase K12 prepared according to the method of Cohen (1951) gave essentially the same results as did nutrient broth.

Cultures of K12 starved on limited glucose for 24 hours demonstrated a lag of approximately one hour when growth was reinitiated in broth, and three hours in M9, as determined by colony counts. This indicates that the resumption of active multiplication is not necessary for the reversal of the phenomenon. Furthermore it indicates that abortive infection does not depend simply on the presence of some cells that are incapable of further multiplication.

The tests of some hypotheses concerning abortive infection. To test the possibility of genetic variability in the bacterial population, twenty-five colonies were picked from nutrient agar plates and tested for abortive infection by the usual method. All of the isolates demonstrated abortive infection although a wide variation in the extent of abortive infection from culture to culture was noted despite the use of the same T2 preparation. Abortive infection seems, therefore, not to be a function of the accumulation of stable mutant bacteria.

Twelve phage cultures were prepared from single T2 plaque isolates. All demonstrated abortive infection. This tends to rule out the involvement of genetic variability among the phage particles and is supported further by the observed variation of the extent of abortive infection between bacterial cultures infected with the same phage suspension.

If a fraction of the phage population were incapable of culminating the infection process when adsorbed on starved bacteria, then this could be overcome by increasing the multiplicity of infection until every infected bacterium was associated with at least one normal phage particle. In a similar way, suppose that the various adsorptive sites of starved bacteria were of at least two sorts—one sort which would permit successful phage penetration and growth and another which would not. In this case also, raising the multiplicity should raise the probability that at least one "effective" site of every infected bacterium is occupied, provided that occupation of an ineffective site does not prevent subsequent

TABLE 3

Abortive infection at higher multiplicities

T2 CONCENTRATION IN ABSORPTION MIXTURE	FRACTION OF FREE T2 AFTER ADSORPTION	FRACTION OF BACTERIA SURVIVING	MULTIPLICITY OF INFECTION BASED UPON FREE PHAGE	FRACTION OF INFECTED BACTERIA FORMING PLAQUES
2.20×10^9	0.120	0.165	1.9	0.460
1.65×10^9	0.142	0.218	1.4	0.544
1.10×10^9	0.150	0.396	0.93	0.459
0.55×10^9	0.073	0.634	0.50	0.527
2.20×10^7	0.105	—	0.010	0.492

Bacterial concentration in all adsorption mixtures was 1.01×10^9. The bacteria were starved for five hours before infection. The data are based upon platings done in quadruplicate.

adsorption and growth of a particle on an effective site.

Table 3 gives the results of an experiment designed to test these hypotheses. Since Weigle and Delbrück (1951) have reported lysis from without at moderate multiplicities with T5 on K12, the multiplicity was not increased above two in this experiment. In other experiments the multiplicity has been carried as high as five without significantly changing the results of the experiment. The adsorption time was kept uniform.

The data indicate that the fraction of infected bacteria which form plaques is not affected significantly by the multiplicity of infection. We can conclude therefore that abortive infection is not due to variability in the phage population or to heterogeneity among the adsorption sites.

These results suggest a nongenetic variability in the bacterial population. The simplest supposition which we can propose is that a certain number of bacteria in any culture are capable of adsorbing phage after starvation but incapable of producing a yield of phage progeny, while the remaining bacteria are capable of adsorption and reproduction.

Lysis of abortively infected bacteria. The turbidimetric changes occurring after infection of starved and unstarved cells (from a single batch of M9 grown cells) by T2 were followed. Multiplicities of infection between two and four were used. Lysis was found to start prematurely and to proceed more slowly in the case of starved cells than unstarved cells. The final levels of lysis were essentially the same in both cases when lysis was followed long enough.

The effect of starvation on the formation of the latent phage in strain K12. Strain K12 is lysogenic (Lederberg and Lederberg, 1953) and carries the latent phage lambda (λ). The demonstration that λ reproduction in strain K12 can be stimulated by irradiation with ultraviolet light (Weigle and Delbrück, 1951) allows the comparison of the synthesis of λ in starved and unstarved cells.

Various doses of ultraviolet light were employed. The induced plaque formers were titrated by plating the irradiated cell suspension in the presence of the λ sensitive strain of strain K12 called Sens (Weigle and Delbrück, 1951). The efficiency of plaque formation by ultraviolet inactivated K12 previously grown in synthetic medium was found to be less than that reported for broth grown cells (Weigle and Delbrück, 1951) when the comparison was made at equivalent levels of bacterial survival. Starved cells also showed an increase in ultraviolet resistance which was not consistent but of an order of magnitude much lower than that reported by Borek and Rockenbach (1952). This might be due to the fact that the bacteria were removed routinely from the starvation medium and resuspended in buffer before irradiation. It was observed that the amount of resistance had some correlation with the amount of the original starvation medium carried over before irradiation.

Starved cultures were found to be induced to synthesize λ with the same efficiency as cultures subjected only to washing when the comparisons were made at equivalent levels of bacterial survival. It therefore seems that the ability to synthesize λ is independent of starvation and depends only on the effective ultraviolet dose (in terms of per cent bacteria inactivated). The relation of ultraviolet resistance to abortive infection is obscure.

The effect of antiserum on adsorbed phage. Another interesting aspect of the adsorption of T2 on strain K12 is brought to light when the number of infected bacteria capable of supporting phage growth is determined by the use of anti-T2 antiserum. Supposedly, once the phage particle becomes attached to the bacterium, it can no longer be inactivated by antiserum (Benzer *et al.*, 1950). Therefore one should obtain the same number of infected bacteria capable of supporting phage growth by measuring the number of infectious centers surviving antiserum treatment as obtained by the centrifugation method employed throughout these experiments.

We have observed repeatedly in experiments done at low multiplicity of infection that if one measures the number of infected bacteria as a function of time in the adsorption mixture, the values obtained with the two methods do not agree. During the early stages of adsorption the number of infected bacteria, as determined by the antiserum method, is substantially lower than that obtained by centrifugation. As adsorption proceeds, the number obtained by antiserum resistance more closely approximates the number obtained by centrifugation. Figure 3 illustrates the results of such an experiment. We can conclude that some process proceeds in buffer at 37 C that renders the adsorbed phage particle immune to the action of antiserum. Lieb (1953) also

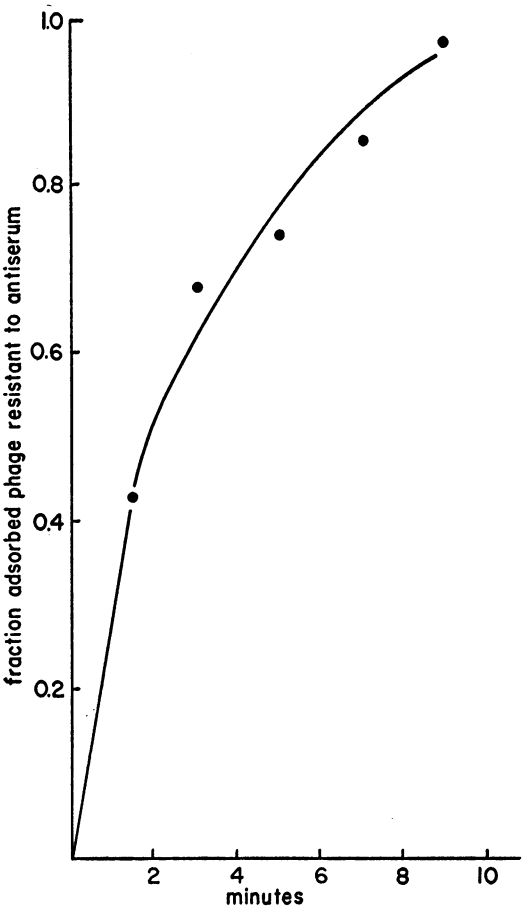

Figure 3. The inactivation of the adsorbed phage particle by antiserum. The ratio of infected bacteria, as determined by the inactivation of phage by antiserum, to infected bacteria, as determined by the centrifugation technique, as a function of time at 37 C in the adsorption mixture.

has found this to be true for λ when it is adsorbed on strain K12 Sens. This seems to provide further evidence for two separate early processes in phage adsorption: one step being virus attachment to the cell surface (perhaps corresponding to the electrostatic attachment postulated by Garen and Puck, 1951), the other step resulting in the resistance of the infecting particle to neutralization by antiserum.

DISCUSSION

The data presented here suggest that the loss of adsorbed phage particles as plaque forming entities is due to a differentiation of the starved K12 population into two classes, one that can support the synthesis of phage when placed in nutrient medium after infection, and another class that can adsorb phage but in which the normal cycle of virus growth is blocked. The determination of bacterial survivors under conditions of multiple infection clearly indicates that all infected cells are inactivated by adsorbed phage. Furthermore, cells which are incapable of supporting phage synthesis are lysed nevertheless after the absorption of phage particles. Despite this it cannot be assumed that, for lysis in abortive infection, the phage must penetrate beyond the initial stages of attachment. The data of Herriott (1951) indicate that T2 ghosts obtained by osmotic shock can adsorb onto and lyse *E. coli*, strain B, and Hershey and Chase (1952) have clearly demonstrated that these ghosts do not get beyond the surface of the cell.

Two early steps of the infective process have been shown. One step is the attachment of the phage particle in such a way that leaves it still sensitive to antiserum; the second, the acquisition of resistance of the invading particle to antiserum. This criterion cannot be applied to determine the extent of invasion of abortively infective particles because the detection of antiserum resistance depends upon the ability of an infected bacterium to support phage growth. It is therefore impossible at this time to associate any of the known early steps of virus penetration with the site of the block in abortive infection. Furthermore, the fact that starved and unstarved strain K12 can be induced to synthesize λ with the same efficiency at equivalent ultraviolet dose levels indicates that the block may not be associated with the ability of the cells to support the synthesis of phage. The site of the block in the life cycle of the phage is, therefore, still obscure. It would seem, however, that a more direct approach to this problem would be a study of the fate of labeled phage material in abortive infection.

A short incubation of starved cultures in a growth medium before infection was found to obliterate the effect of starvation. The association of abortive infection with a biochemical deficiency is hard to determine since the addition of broth or bacterial extracts to starved bacteria at the onset of adsorption had no effect on the number of infectious centers recovered. It would seem then that the reversibility of abortive infection is associated in some way with the physio-

logical changes that eventually lead to bacterial multiplication.

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SUMMARY

A large proportion of the phage particles adsorbed on starved cells is lost as infectious centers. This phenomenon, called "abortive infection", probably is due to the incapability of a fraction of the bacterial population to support phage synthesis.

The loss of capability of starved cells to support phage growth can be reversed by incubating them in growth medium for 30 minutes before infection. This reversibility has been shown not to depend on the adsorption of phage on multiplying bacteria, though multiplying bacteria are not subject to abortive infection.

Some resistance to the bactericidal effect of ultraviolet light was noted in starved cultures although the frequency of induced lambda formation was found to be the same in starved and unstarved cultures at doses resulting in equivalent levels of bacterial survival.

At an early stage in the adsorptive process, the adsorbed phage particle is sensitive to inactivation by antiserum.

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