A REVERSIBLE TRANSFORMATION OF T1 BACTERIOPHAGE[1]

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In the course of a study of the mechanism of adsorption of T1 bacteriophage on *Escherichia coli*, strain B, an unexpected destruction of the virus was observed in repeated experiments when it was added to susceptible cells under conditions that should have led to normal adsorption. Analysis of this situation led to the conclusion that T1 bacteriophage constitutes a system in which at least two forms can coexist in equilibrium. These forms differ markedly in their reaction with susceptible cells. In the present paper, the existence of this reversible transformation is established, and preliminary experiments on the properties of the two forms are described.

METHODS AND MATERIALS

T1 bacteriophage and *E. coli* B were used in all the experiments. Each day a fresh dilution was prepared from a single broth lysate containing 1×10^{10} particles per ml, which was stored in the refrigerator. The activity of the stock solution was checked frequently and found to be constant throughout the period of study. Difco dehydrated nutrient broth plus 0.5 per cent NaCl was used as the growth medium. All plaque counts were derived from platings in nutrient agar made by the standard technique (Delbrück, 1942). Aliquots of the virus solution were added to 2.0 ml of soft nutrient agar at 45 C, containing 0.05 ml of a 24-hour culture of *E. coli* B grown in aerated, nutrient broth. The mixture was poured immediately on nutrient agar plates and incubated at 37 C. All plaque counts were performed in duplicate with a resulting precision of about 10 per cent.

EXPERIMENTAL RESULTS

The initial observation arose out of an attempt to compare the adsorption rates of T1 on *E. coli* B in broth and distilled water, respectively, in order to determine whether cofactor molecules take part in this reaction (Anderson, 1948*b*). The standard T1 preparation was diluted in distilled water,[2] and added to each

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[2] The experiment presented some difficulty due to the fact that T1 tends to be unstable in distilled water at 37 C. However, by expeditious manipulation, the total time of sojourn of the virus in water before its addition to the adsorption tubes could be reduced below 5 minutes and under these circumstances anywhere from 10 to 75 per cent of the virus remained viable so that the experiment was feasible.

of two tubes, one containing a concentrated culture of *E. coli* B in broth and the other an equal volume of the same cells redispersed in distilled water. At measured time intervals, samples were removed for the determination of the proportion of the virus that had become attached to cells. When aliquots from two such suspensions were plated, the unexpected result was obtained that almost no viable virus remained in the broth suspension, although a fairly good recovery was obtained from the mixture of virus and cells in distilled water.

TABLE 1

Loss of activity of virus diluted in water when added to cells in broth

PLAQUE COUNTS FROM SUSPENSION OF CELLS PLUS VIRUS IN WATER	PLAQUE COUNTS FROM SUSPENSION OF CELLS PLUS VIRUS IN BROTH
406	0

Two 6-ml samples of a 3-hour, aerated culture of *E. coli* B in nutrient broth were centrifuged and resuspended in 1.2 ml of the original broth supernatant and in 1.2 ml of distilled water, respectively. The concentration of *E. coli* in each tube was about 10^{10} per ml. To each tube, maintained in a 37 C bath, was added 0.1 ml of a $1:10^4$ dilution in water of the standard broth T1 lysate. After 4 minutes both adsorption tubes were plunged into an ice bath to stop further action, and 0.1-ml quantities from each tube were plated in nutrient agar seeded with *E. coli*.

TABLE 2

Demonstration that extensive phage destruction occurs only when virus diluted in water is added to cells in broth

VIRUS DILUTION MEDIUM	PER CENT OF THEORETICAL PHAGE ACTIVITY RECOVERED WHEN ADDED TO CELLS SUSPENDED IN	
	(A) Broth culture fluid	(B) H_2O
(1) H_2O	7%	64%
(2) Broth	100%	100%

The procedure was similar to that in table 1. The somewhat lower count in tube 1B with respect to that in 2B is due to the lower stability of the virus when diluted in water at 37 C.

This result was particularly surprising because T1 tends to decompose at 37 C in distilled water but is ordinarily quite stable in nutrient broth. Table 1 illustrates a typical experiment.

When each step of this procedure was examined, the critical point was found to lie in the preliminary dilution of the virus in H_2O, for, when the original phage lysate was diluted with nutrient broth before its addition to the two cell suspensions, no loss of plaque formation occurred in either tube. This is shown in the experiment of table 2.

For further study of this action, a lower temperature was resorted to in order to minimize the inactivating effects on T1 of solutions of low ionic strength (Adams, 1949). The following standard procedure was adopted: The original

broth lysate of T1 was diluted 1:10^4 or more in various test media maintained at 2.0 C, and was kept at this temperature for 15 minutes or more in order to achieve equilibration between the virus and the solution. Aliquots were then removed and added to cell suspensions at 15 C. The first step was conducted at 2.0 C because the virus is most stable at low temperatures; the second was performed at 15 C because that is the lowest temperature at which T1 is adsorbed on cells with a velocity great enough to be measured conveniently. It was found necessary to use doubly distilled water for the preparation of the equilibrating solutions, as impurities in ordinary distilled water were sufficient to produce erratic results. In order also to eliminate any effects due to the accumulation of bacterial metabolic products within the broth culture filtrates, the practice was adopted of resuspending all cells in fresh medium before the addition of the virus.

TABLE 3

Experiment demonstrating that the presence of bacterial cells is necessary for the destruction of T1 virus that has been sensitized by a previous equilibration in cold phosphate buffer

TUBES TO WHICH EQUAL AMOUNTS OF SENSITIZED VIRUS WERE ADDED	CONCENTRATION OF BACTERIA IN EACH TUBE	PLAQUE COUNTS AFTER A 5-MIN SOJOURN IN EACH TUBE
(1) Broth, 15 C	9.4×10^8/ml	62
(2) Broth, 15 C	0	920

The standard broth suspension of T1 was diluted 1:10^4 in 2×10^{-4} M phosphate buffer of pH 7.5 at 2.0 C, and kept at this temperature for 15 minutes. One-tenth-ml quantities of this solution were then added to each of 2 tubes maintained at 15 C, the one containing 0.9 ml of broth only and the other 0.9 ml of a broth suspension of washed and concentrated young *E. coli* cells. Five minutes after the addition of the virus, aliquots were removed from each tube and plated. The theoretical plaque count to be expected if no virus destruction had occurred is 1,200.

At lower temperatures the sensitizing action of a preliminary sojourn in distilled water that causes subsequent inactivation of the virus when added to host cells in broth was confirmed. Dilute phosphate buffer (pH 7.5) behaved like distilled water in this respect and was a more satisfactory diluent because it produced less virus destruction during the initial equilibration period.

No inactivation occurs when sensitized virus is added to broth, unless the cells are also present, as shown in table 3. When cell suspensions of varying density were employed, the loss of activity increased directly with the cell concentration and approached 100 per cent for bacterial suspensions of about 10^9 per ml or greater, indicating that a 15-minute pre-equilibration period in phosphate buffer was sufficient to convert practically all the virus to the sensitized state.

Role of inorganic ions. The possibility that sensitization might depend on small amounts of inorganic ions was suggested by the fact that in an occasional experiment with a phosphate buffer stock solution that had been in use for some weeks sensitization failed to occur. Repetition of the procedure with a freshly

prepared solution caused the reappearance of the phenomenon. Further tests showed that the addition of $CaCl_2$ to the equilibrating solution could protect the virus against sensitization. This action is illustrated in the data of table 4. Concentrations of $CaCl_2$ as high as 5×10^{-3} M worked just as well, but little effect was obtained from solutions as dilute as 10^{-5} M.

Nature of the virus-destroying process. This inactivation of sensitized phage arises from its interaction with the bacterial cells themselves rather than with any products of the cell metabolism. Thus, sensitized virus, added in parallel tests to a broth suspension of bacterial cells and to the supernatant from an identical suspension immediately after removal of the cells by centrifugation, was inactivated only in the tube containing bacteria. Moreover, when the cells that had been separated from the original suspension were redispersed in fresh broth and tested promptly thereafter with another sample of sensitized virus, plaque formation was again sharply depressed. This behavior can be explained

TABLE 4

Prevention of sensitization by the presence of $CaCl_2$ in the equilibrating mixture

MEDIUM USED TO DILUTE AND EQUILIBRATE PHAGE AT 2.0 C	CELL CONCENTRATION IN EACH BROTH TUBE AT 15 C, TO WHICH VIRUS WAS SUBSEQUENTLY ADDED	PLAQUE COUNTS	DESTRUCTION IN TUBE WITH CELLS AS COMPARED WITH CONTROL CONTAINING NO CELLS
I. 0.005 M phosphate buffer	(a) 2×10^9/ml	29	88%
	(b) 0	248	
II. 0.005 M phosphate buffer plus 10^{-4} M $CaCl_2$	(a) 2×10^9/ml	402	0
	(b) 0	404	

T1, from each of the two equilibrating solutions shown, was added (a) to a suspension of cells in broth and (b) to plain broth at 15 C. Ten minutes later samples from each of these 4 tubes were removed and plated.

by assuming that inactivation of sensitized virus is a result of its adsorption on cells while in a state in which subsequent steps of its metabolic cycle are blocked. Since adsorption is practically irreversible (Schlesinger, 1932), such phage particles would not form plaques. Virus remaining unadsorbed could form a plaque if it were reconverted to the "normal" form by the nutrient agar plating medium.

To test this picture, simultaneous measurements of the rates of adsorption and destruction of sensitized virus were made. It was found that, within the limits of the experimental procedure, the two rates were identical. Hence the presumption is supported that if virus is adsorbed on cells while in the sensitized state, it loses its ability to form a plaque on subsequent plating. Table 5 describes such an experiment in which normal and sensitized virus were each added to cell suspensions in broth. Extensive loss of activity occurred only in the tube containing sensitized virus and cells. Yet the titer of free virus in this tube (Ia) closely paralleled that in the companion tube containing normal virus and cells (IIa) in which no loss of activity occurred.

If this interpretation of the underlying process is correct, the percentage of

destruction of adsorbed sensitized virus may be simply calculated. For example, in the experiment of table 5, during the first 5 minutes:

sensitized T1 adsorbed = 686 − 70 = 616 or 90 per cent of the total

sensitized T1 adsorbed and viable = 175 − 70 = 105

$$\text{Fraction of adsorbed virus killed} = 1 - \frac{105}{616} = 83 \text{ per cent}$$

In several repetitions of this experiment, the rate of disappearance of free virus from broth cell suspensions was the same for either normal or sensitized preparations and always obeyed the linearly logarithmic rate characteristic of virus adsorption (Schlesinger, 1932; Delbrück, 1940). The adsorption constants calculated from these experiments are summarized in table 6. The es-

TABLE 5

Measurement of total virus activity and the amount remaining unattached to cells when normal and sensitized virus preparations are each added to cells in broth at 15 C

VIRUS PREPARATION	CONCENTRATION OF *E. coli* IN THE ADSORPTION TUBE	PLAQUE COUNTS			
		After 5 minutes		After 10 minutes	
		Total virus (free and cell-attached)	Supernatant virus (free virus only)	Total virus	Supernatant virus
I. Sensitized T1 (equilibrated in phosphate buffer at 2.0 C)	(a) 1.23×10^9	175	70	154	18
	(b) 0	686			
II. Normal T1 (equilibrated in phosphate buffer plus 10^{-3} M $CaCl_2$)	(a) 1.75×10^9	920	74	1,042	26
	(b) 0	972			

Equal amounts of virus were used in each case. The lower titer in the control tube with sensitized virus (686 instead of 972) is due to the somewhat poorer survival of T1 in phosphate buffer without Ca^{++} ion, during the equilibration period.

sential constancy of these values would indicate that adsorption of sensitized phage on susceptible cells proceeds at the same rate as for normal virus, even though in the former case most of the resulting infections cannot reproduce free virus.

Behavior of sensitized virus toward cells in distilled water. The foregoing conclusions would be strengthened if it were possible to show that when sensitized virus is added to host cells under conditions in which no adsorption occurs, inactivation is also prevented. Such behavior is achieved in distilled water. Sensitized virus does not lose its ability to form plaques when added to cells in distilled water (tables 1 and 2). Analysis of the supernatant phage content of such mixtures revealed that adsorption also fails to occur in this medium (table 7).

When a synthetic medium consisting of 0.02 M glucose, 0.015 M $(NH_4)_2SO_4$, 0.02 M phosphate buffer, and 10^{-3} M $MgSO_4$ was substituted for distilled water,

TABLE 6

Demonstration that the rate of removal of free, sensitized virus from a broth suspension of cells is the same as that of normal virus

VIRUS TREATMENT BEFORE ITS ADDITION TO CELLS IN BROTH AT 15 C	CONCENTRATION OF *E. coli* IN ADSORPTION TUBE	k = ADSORPTION CONSTANT	ADSORBED VIRUS INACTIVATED
A. *Sensitized:*			
Equilibrated in H_2O or phosphate buffer at 2.0 C	6.0×10^8	3.0×10^{-10}	54%
	1.1×10^9	3.0×10^{-10}	51%
	1.2×10^9	3.7×10^{-10}	83%
	2.0×10^9	4.8×10^{-10}	88%
	3.2×10^9	1.0×10^{-10}	86%
B. *Normal:*			
Equilibrated in phosphate buffer plus $Ca^{++}(10^{-3}$ M) at 2 C	1.8×10^9	2.9×10^{-10}	0
	1.8×10^9	3.6×10^{-10}	0
	2.0×10^9	2.3×10^{-10}	0
C. *Normal:*			
Equilibrated in nutrient broth	2.1×10^9	1.9×10^{-10}	0
	2.1×10^9	2.1×10^{-10}	0
	1.4×10^9	1.9×10^{-10}	0
	1.4×10^9	2.0×10^{-10}	0

The concentration of free, viable phage, P, present at any time, t, measured in minutes, can be calculated from:

$$2.303 \text{ Log } \frac{P}{P_0} = -kBt$$

where P_0 is the initial concentration of phage at the moment of addition of the cells, B is the bacterial concentration, and k is the adsorption constant. The percentage of adsorbed virus inactivated was calculated as in the experiment of table 5.

TABLE 7

Experiment demonstrating that in the absence of adsorption, no inactivation of sensitized virus occurs

MEDIUM IN ADSORPTION TUBE	*E. coli* CONCENTRATION	5-MINUTE PLAQUE COUNTS		10-MINUTE PLAQUE COUNTS	
		Total suspension	Supernatant	Total suspension	Supernatant
(a) H_2O (double-distilled) 15 C	3×10^9/ml	1,156	938	995	926
(b) Nutrient broth, 15 C	3×10^9/ml	359	227	188	84

Equal amounts of sensitized T1 were added to both tubes, a and b. Neither adsorption nor loss of plaque-forming ability occurred in the distilled water tube; both processes occurred in the broth medium.

the capacity of T1 to be adsorbed on *E. coli* B at 15 C was restored. The rate was linearly logarithmic and equal to that which is obtained in broth at the same temperature. As was expected, when sensitized T1 phage and cells were brought together in this medium, extensive inactivation with loss of 90 per cent of the plaque-forming titer occurred.

The evidence that the phage inactivation described is a result of adsorption of sensitized virus on host cells may be summarized: The loss in activity requires direct interaction between the sensitized phage and the cells. When this inactivation occurs in a phage-cell mixture, the rate of disappearance of free, viable phage is the same as in a suspension in which normal phage is adsorbed on cells without inactivation. If sensitized phage and cells are brought together in distilled water in which adsorption does not occur, inactivation also does not take place. Finally, when the ability of the phage to absorb is restored to such a solution by the addition of inorganic salts, the characteristic inactivation also returns.

Reversal of sensitization. Sensitized virus can be restored to the normal form by a sojourn in broth, if it has not been adsorbed on cells. Detailed study of the rates of the forward and backward reactions at various temperatures has not yet been made, but preliminary experiments have been conducted in which recovery was tested by adding a large amount of cells to tubes containing sensitized T1 that had had varying recovery periods in broth at 15 C. The longer

TABLE 8

Reversal of sensitization in broth at 15 C

TUBE	RECOVERY PERIOD IN BROTH AT 15 C BEFORE ADDITION OF CELLS	PLAQUE COUNT AFTER A 5-MINUTE ADSORPTION PERIOD AT 15 C	PER CENT OF ORIGINAL VIRUS ACTIVITY THAT WAS VIABLE
A	0	278	34
B	16 minutes	726	89

Equal amounts of a sensitized T1 preparation were added (A) directly to a broth cell suspension, and (B) to a tube of plain broth to which an equal number of cells was added 16 minutes later. Each tube was sampled 5 minutes after the virus and cells had been brought together. The final bacterial concentration in each tube was 3.5×10^8 per ml.

the sojourn in broth before the cell addition, the greater was the number of plaques formed on subsequent plating. A sample experiment is shown in table 8, in which almost complete recovery had occurred after 16 minutes. A few experiments at higher temperatures indicated that recovery is more rapid at 37 C than at 15 C.

DISCUSSION

Anderson (1948*a*) discovered a reversible transformation in strains of T4 bacteriophage, the effect of which was to make adsorption impossible in the absence of trypotophan, and Delbrück (1948) later described another T4 strain requiring both tryptophan and Ca^{++} for adsorption. The experiments of the present paper demonstrate a transformation which is also reversible under certain conditions, but which blocks some step occurring after adsorption. Sojourn in water or dilute phosphate buffer apparently converts T1 to a form which can be adsorbed at exactly the same rate as the parent form, but which cannot lyse the infected cell and produce new free virus. Moreover, after adsorption the virus activity appears to be permanently lost.

The existence of different equilibrium states of the virus may be necessary for the performance of different steps of its metabolic cycle. When, as in T1, such transformations are reversible only during a part of this cycle, possibilities arise for chemical distortion of the normal multiplication pattern. These experiments also suggest that different functions are localized at different sites within the virus particle, since by adjustment of the chemical environment certain activities can be blocked while others proceed normally.

The kinetics and specificity of the Ca^{++} ion in controlling the degree of sensitization of T1 remain to be investigated. The fact that phosphate buffer solutions after some weeks of use sometimes lose the ability to sensitize indicates possible effects of minute quantities of ions that might be introduced as impurities on glassware. Study with highly purified reagents of the action of a variety of ions is planned.

Behavior involving adsorption of phage without reproduction has been reported by Andrewes and Elford (1932), who found an *E. coli* bacteriophage which was rendered incapable of multiplication in the presence of citrate, but which could still react with bacteria so as to prevent subsequent multiplication of the cells; and by Luria and Delbrück (1942), who induced a similar action in T2 phage by exposure to small doses of ultraviolet light. The adsorption reaction of bacteriophage appears to be less sensitive to ultraviolet radiation or to an unfavorable ionic environment than other phases of its metabolic cycle.

Adams (1949) has recently studied the irreversible inactivation of *E. coli* bacteriophages occurring in the absence of cells in solutions of low ionic strength, and was led to postulate a reversible dissociation between the virus particle and metallic cations like Ca^{++} as an intermediate step in a series of reactions leading to the destruction of the phage. The sensitized phage described here may be the cation-dissociated intermediate postulated by Adams. An unpublished previous observation has also been communicated by Adams, describing a metabolic block in the reproduction of T5, produced by a lack of Ca^{++} ions. This effect differed from sensitization of T1 in that it could be reversed by the subsequent addition of the Ca^{++} ion to the infected cell, but it may represent an analogous phenomenon.

SUMMARY

In distilled water or dilute phosphate buffer, T1 bacteriophage undergoes a transformation to a sensitized form that loses its ability to form plaques if it is immediately added to a suspension of host cells in broth.

This transformation can be reversed by allowing such sensitized phage to recover in plain broth before the addition of the cells, in which case almost the theoretical number of plaques is obtained when the mixture is plated.

Sensitization could be prevented by $CaCl_2$ in the equilibration medium in a concentration of 10^{-4} M or higher.

Inactivation of sensitized phage involves direct interaction with host cells and only occurs under conditions that allow adsorption of the phage. The rate of this inactivation is the same as the rate of adsorption of normal phage. It

is concluded that sensitization produces a block in some step of the virus metabolic cycle occurring after adsorption. The act of adsorption renders this transformation irreversible causing loss of the virus activity.

The strain of T1 phage employed is not adsorbed on host cells in distilled water at 15 C. Adsorption does take place in a solution of glucose and inorganic salts, and the rate is exactly the same as in broth at the same temperature.

The significance of the ability of bacteriophage to undergo reversible transformations to forms with different metabolic properties is discussed.

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