THE ACTIVATION OF THE BACTERIAL VIRUS T4 BY L-TRYPTOPHAN[1]

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A few years ago I discovered that preparations of the bacterial viruses T4 and T6 produce more plaques when plated with their host, *Escherichia coli* strain B, on Difco nutrient agar (N) than they do on agar containing only inorganic salts and ammonium lactate as a source of nitrogen and carbon (Anderson, 1945*b*). It, therefore, appeared that some substance or cofactor, present in the nutrient medium, had promoted the activity of these viruses. To identify the activating substance the effect on virus activity of individual growth factors and amino acids was studied. L-Tryptophan was found to be most active in promoting plaque formation by T4, whereas phenylalanine, diiodotyrosine, and tyrosine showed progressively lower activities. Many other synthetic amino acids having aromatic groups are also active, but D-tryptophan appears not to be active (Anderson, 1946*a*,*b*). Oddly enough, norleucine is slightly active (Delbrück, 1948).

Regarding the mechanism of action of these substances it was discovered that T4 and T6 particles were not even adsorbed on host cells in the synthetic medium (Anderson, 1945*b*). This result, first obtained by centrifugation experiments, is strikingly confirmed by electron microscope studies of virus-host mixtures with and without added tryptophan. In the synthetic F medium no T4 particles are seen adsorbed on the host cells (figure 1, no. 1), but on the addition of tryptophan to the mixture many tadpole-shaped virus particles may be seen attacking the cell surface (figure 1, nos. 2A,B) (Anderson, 1946*b*). In some way, therefore, L-tryptophan has the power of promoting the *adsorption* of the virus T4 on its host. We shall refer to compounds having such action as *adsorption cofactors*.

Because this phenomenon offers an experimental tool for the study of the specific, but little understood, mechanisms of virus adsorption, it was decided to investigate the matter further—in particular to determine the site of action of the cofactors. This work, which is reported here, shows that it is the virus particles that are activated by L-tryptophan rather than the bacterial cells.

MATERIAL AND METHODS

The host (*Escherichia coli* strain B), the virus T4, the growth media, and the methods of assay were the same as those reported earlier (Anderson, 1945*b*).

Virus was grown on the host in 1,500-ml shaker flasks, with aeration at room

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temperature, in the synthetic ammonium lactate medium (F). It was then purified and concentrated by differential centrifugation: 4,000 rpm for 20 minutes to remove the unlysed bacteria and 10,000 rpm for 2 hours to sediment the virus. The virus so obtained was resuspended in F medium, and after a few days' or weeks' storage at 4 C the differential centrifugation was repeated. Finally, the material was filtered through a Mandler filter of "medium" porosity to remove bacterial contaminants.

Two such virus concentrates were used in this work. One, designated T4 (5/10/45), was obtained by seeding the shaker flask containing B with about 10^6 T4 particles from a clone derived from the original T4 stock. This concentrate assayed about 1.6×10^{11} on nutrient (N) agar and about 3×10^8 on F agar. The other T4 concentrate was obtained by seeding a shaker flask containing B with T4r plaque (Hershey, 1946) isolated from an N agar plate used in a routine assay of the T4 (5/10/45) concentrate. This stock concentrate, designated T4r (3/10/47), gave an assay of 3×10^{11} particles per ml on N agar and only 6×10^5 on F agar.

Two methods were available for the determination of the relative concentrations of activated virus, T4*. One method takes advantage of the fact that once the virus particles have become attached to host cells the infected cells are efficient in producing plaques on bacterial smears on F agar at 37 C. The number of plaques observed on this medium is thus taken to be proportional to the concentration (B:T4) of bacteria that had been infected under the conditions of adsorption of the virus on the host. The total concentration (T4) of plaque-forming particles will be taken to be proportional to the number of plaques that are formed by appropriate dilutions on Difco nutrient agar (N) or on F agar containing 10 μg L-tryptophan per ml (Anderson, 1945*b*).

Another method involves the phenomenon of summary lysis (Anderson, 1945*a*). When the host cells are heavily irradiated with ultraviolet light and then exposed to T4 virus in the presence of L-tryptophan, they are rapidly lysed without the multiplication of the virus. The rate and extent of lysis was found to be a qualitative measure of the amount of T4* in the mixture of virus and irradiated B.

FIGURE 1

Procedure: E. coli strain B grown for 18 hours at 20 C on a synthetic medium (F) slant was suspended in 1 ml of liquid F medium, and 0.1-ml samples were placed in test tubes numbered 1 and 2. One-tenth ml of T4 virus suspension in synthetic (F) medium was added to test tube no. 1 and 0.1 ml of T4 virus in synthetic (F) medium containing 100 μg L-tryptophan per ml was added to test tube no. 2. The tubes stood 3 minutes at 25 C. A droplet from each test tube was then placed on a separate formvar membrane. After the membranes had stood 30 seconds, they were washed 8 times in distilled water and allowed to dry. The specimens were then placed in the electron microscope holders, shadowed with gold (Anderson, 1946*c*), and examined in an RCA type B electron microscope with no limiting objective aperture.

No. 1. *E. coli* B + T4 virus from test tube no. 1 containing no added cofactor. No adsorbed virus is visible. EMG 213d × 10,000.

No. 2A. *E. coli* B + T4 virus from tube no. 2 containing 50 μg L-tryptophan per ml. Many virus particles are adsorbed on each host cell. EMG 216d × 10,000.

No. 2B. *E. coli* B + T4 virus from tube no. 2. EMG 217d × 25,000.

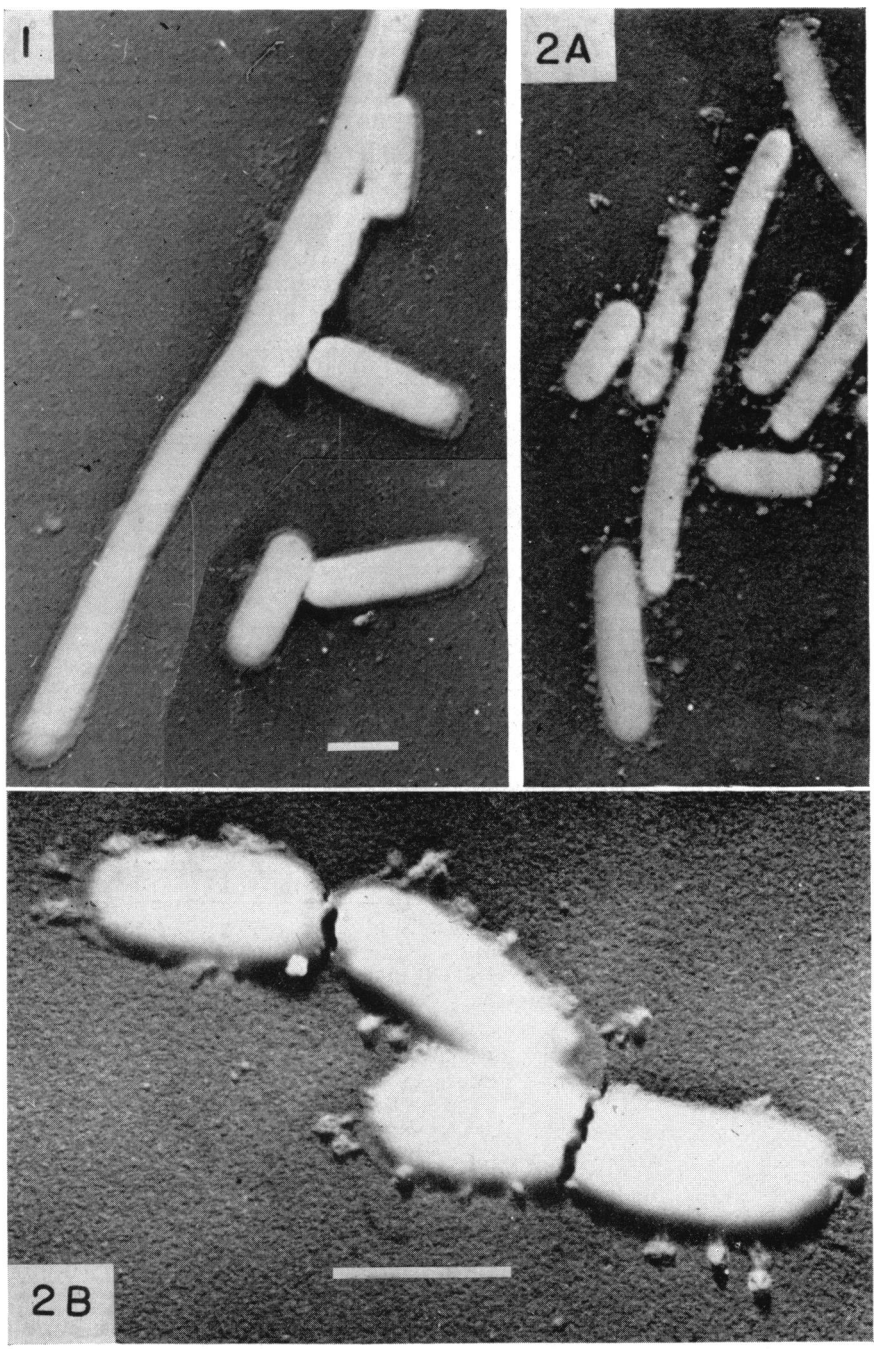

FIGURE 1

EXPERIMENTAL RESULTS

The Dependence of T4 Activity on Tryptophan Concentration

Summary of lysis method. To each of 6 colorimeter tubes was added 0.1 ml of the concentrated suspension of T4 (5/10/45). To each was then added 0.1 ml of F medium containing 14, 10, 6, 4, 2, and 0 μg of DL-tryptophan, respectively. About 5 minutes later, at time t = 0, to the first tube were added 4.0 ml of a suspension of B that had previously been irradiated with ultraviolet light from

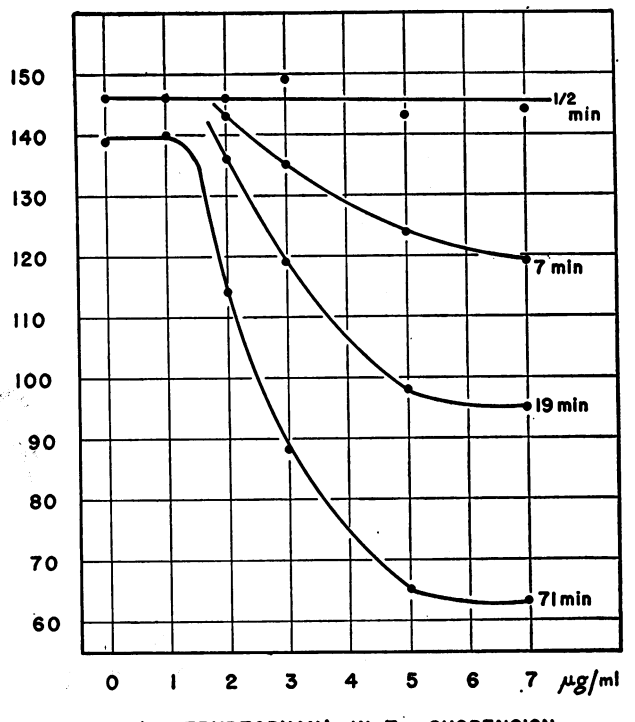

FIG. 2. THE LYSIS OF IRRADIATED B SUSPENSIONS ADDED TO T4 (5/10/45) CONCENTRATES CONTAINING VARIOUS ADDED CONCENTRATIONS OF L-TRYPTOPHAN

The turbidities, T, of the mixtures of bacteria and virus are plotted against the tryptophan concentrations with which the T4 was incubated.

an H-4 lamp (Anderson, 1945*a*). At t = ½ minute the turbidity of this suspension was read with a Klett colorimeter. At t = 1 minute 4 ml of the irradiated B suspension were added to the second tube, its turbidity was read at t = 1½ minutes, and so on until irradiated B had been added to each of the tubes and the turbidities of all the mixtures determined. At later intervals the suspensions were taken in turn, shaken gently to prevent settling of the bacteria, and their turbidities read. The results are presented in figure 2, where the turbidity readings are plotted as ordinates against the concentration of tryptophan in the virus suspension before the addition of the bacteria. Curves have been drawn

through the points for each of the various time intervals. It is seen that the measurable lytic activity increases rather abruptly from nil for virus exposed to less than about 1.5 μg DL-tryptophan per ml to a marked value at 2 μg per ml.

In another experiment it was found that no measurable lysis was induced by adding the same total *amounts* of tryptophan to 4-ml mixtures of virus and irradiated B. These results indicate that the tryptophan activated the virus in the concentrated suspensions, rather than the bacteria after they were mixed with the T4-tryptophan suspensions.

Plaque count method. In a series of preliminary experiments samples of T4 (5/10/45) were incubated with L-tryptophan at various concentrations in F. Ten ml of B in F were then added with stirring to 0.1-ml samples of the T4-tryptophan mixtures. After 5 minutes had been allowed for adsorption, the mixtures were assayed for (B:T4) and for (T4) on F and N agar, respectively. The ratios of the assays on F to the assays on N rose 100-fold for incubation concentrations between 0.2 and 10 μg L-tryptophan per ml. In parallel experiments in which L-tryptophan was added to mixtures of T4 and B in F, a corresponding rise in (B:T4) did not occur until the tryptophan concentrations in the *mixtures* were brought to 0.2 to 10 μg L-tryptophan per ml. Since the concentrations to which the B mixtures were exposed in the first experiments were only 1/100 of these values, whereas those to which the virus had been exposed were the same, it appeared that the tryptophan acted on T4 in making it capable of adsorption rather than on B in making it "receptive" to the virus.

An experiment in which a series of T4r (3/10/47) samples were incubated with *various* tryptophan concentrations and adsorbed on B at the *same* tryptophan concentrations is summarized in figure 3. It is seen that the number of plaques obtained on F agar increased over 100,000-fold as the tryptophan concentrations with which the T4 was incubated increased from 0.1 to 1.0 μg per ml. Since the only systematic variable in the experiment was the tryptophan concentration to which the *virus* had been exposed we may conclude that the effect of tryptophan was on the virus.

Activation of T4 by L-Tryptophan as a Function of Temperature

Summary of lysis method. Samples of T4 (5/10/45) were exposed to 6 μg DL-tryptophan per ml for various lengths of time at room temperature and at 0 C. Irradiated host bacteria were then added to each in turn and the turbidities of each tube read at intervals. The results, plotted in figure 4, show that the temperature of exposure of the virus to this cofactor had a decided effect on the virus activity, little or no activity resulting from exposure at 0 C and marked activity resulting from analogous exposures at room temperature.

Plaque count method. In three experiments, 0.1-ml samples of T4 (5/10/45) containing 20 μg of L-tryptophan per ml were placed in test tubes and incubated at various temperatures for 1½ to 5 hours. To them were then added with stirring 10-ml volumes of actively growing B in F medium at 37 C. The mixtures were then assayed on F agar for (B:T4) and on N agar on F agar containing 10

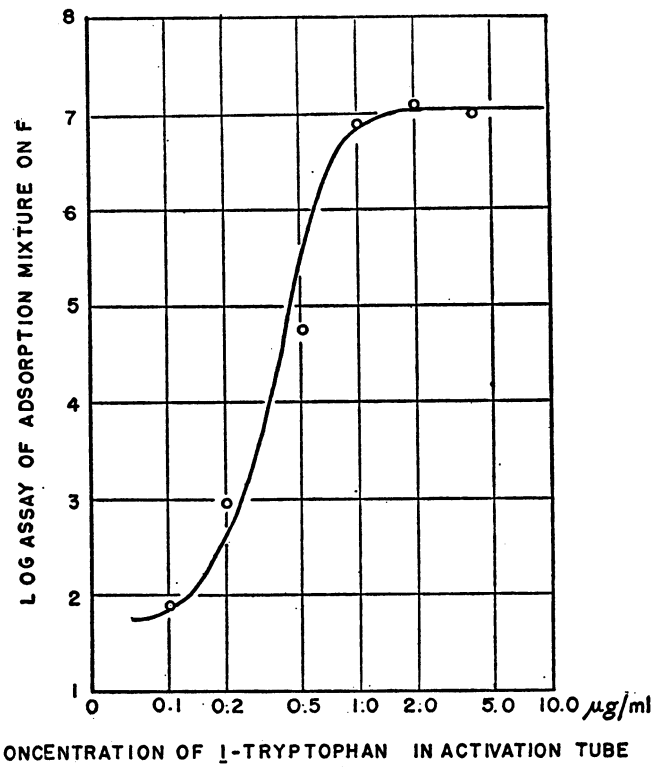

FIG. 3. THE FORMATION OF PLAQUES ON F MEDIUM BY MIXTURES OF HOST BACTERIA AND T4 THAT HAD BEEN ACTIVATED BY VARIOUS CONCENTRATIONS OF L-TRYPTOPHAN

Procedure: T4r (3/10/47) was diluted 1:10 in F medium containing 100 μg of L-tryptophan. Various dilutions of this mixture were then made and volumes placed in a series of incubation tubes (labeled *s* to *x*) such that each tube contained 0.2 μg of L-tryptophan and 6×10^8 virus particles as follows:

TUBE	s	t	u	v	w	x	y
Volume (ml)	2.0	1.0	0.4	0.2	0.1	0.05	0.5
μg tryptophan per ml	0.1	0.2	0.5	1.0	2.0	4.0	0.1

The incubation tubes were kept at 37 C for 25 minutes, at the end of which time the contents of the following bacterial tubes each containing 4×10^{10} bacteria were added to the corresponding tubes of virus.

TUBE	S	T	U	V	W	X	Y
ml 24-hr-aerated B in F	8	8	8	8	8	8	8
ml F medium	0	1.0	1.6	1.8	1.9	1.95	1.8

After 5 minutes had been allowed for adsorption, the mixtures were assayed on F agar and on N agar for B:T4 and for total virus, respectively. The assays on N agar were constant; those on F agar varied as shown. Since the conditions in the adsorption tubes were identical, the differences in the assays on F agar must have been due to the differences in the *concentration* of L-tryptophan with which the virus had been incubated.

μg L-tryptophan per ml for (T4). The results, expressed as ratios between the adsorbed virus and free virus:

$$R = \frac{(B:T4)}{(T4) - (B:T4)}$$

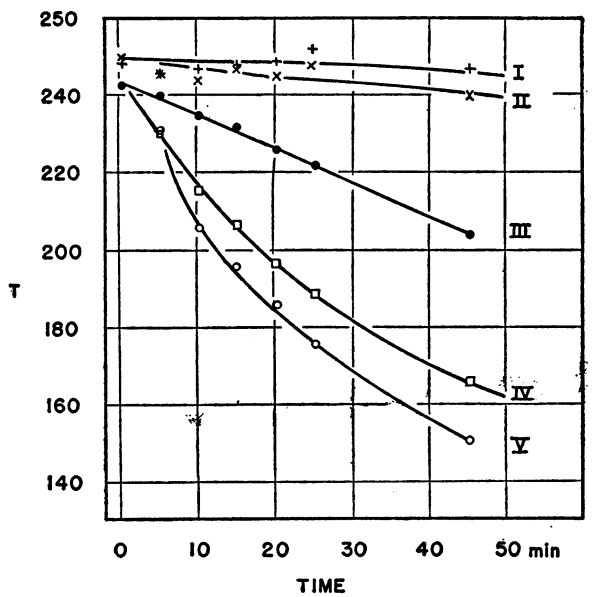

FIG. 4. THE EFFECT OF TEMPERATURE OF INCUBATION ON THE ACTIVATION OF T4 (5/10/45) BY 3 μG L-TRYPTOPHAN PER ML AS DETERMINED BY ITS SUMMARY LYSIS OF UV IRRADIATED HOST CELLS

Procedure: To 0.1 ml samples of T4 (5/10/45) in colorimeter tubes was added 0.1 ml of DL-tryptophan. The tubes were incubated at 0 C and at room temperature as follows:

SAMPLE	μG DL-TRYPTOPHAN/ML	SUCCESSIVE TEMPERATURE TREATMENTS		
		Min at room temperature	Min at 0	Min at room temperature
I	0	0	0	10
II	3	0	10	0
III	3	0	5	5
IV	3	5	5	0
V	3	0	0	10

Four samples of B irradiated 10 minutes with the H-4 arc were then added to each and the turbidities (T) read on a Klett colorimeter and plotted against times after mixing.

are plotted in figure 5 against the reciprocals of the absolute temperatures T at which the virus was equilibrated with tryptophan.

It is seen that the activation is a function of the temperature to which the virus was exposed during contact with cofactor, being a maximum near 35 C and falling off rapidly on either side. The extension of the value to higher temperatures is prohibited by the rather rapid inactivation of the virus above 60 C.

Activation of T4 by L-*Tryptophan as a Function of pH*

Plaque count method. Aliquots of the T4 (5/10/45) suspension were added to F medium and, adjusted to various pH's with HCl or NaOH and enough L-tryptophan was added to each to bring the concentration to 20 μg per ml. After standing at 28 C for 30 minutes, 0.1-ml samples were added with stirring to 10-ml samples of an aerated culture of B in F medium. The latter mixtures were then assayed for (B:T4) on F agar and for (T4) on F agar containing 10 μg of L-tryptophan per ml. The results, expressed in terms of R as before, are plotted in figure 6. It is seen that the activation is again a function of the conditions

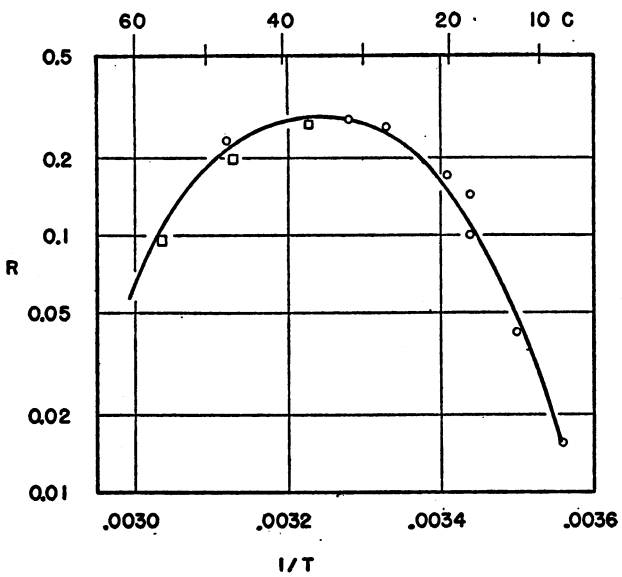

FIG. 5. THE ACTIVATION OF T4 BY 20 μG L-TRYPTOPHAN PER ML AT VARIOUS TEMPERATURES

The results of three experiments are plotted. The ratios R of adsorbed to free virus are plotted as ordinates against the reciprocal of the absolute temperature of activation as abscissae.

of exposure of the virus to tryptophan, being a maximum at pH 7.5 and falling off on the acid side. Above pH 8.6 and below pH 3.8 the virus is rapidly inactivated in F medium so the extension of the curve beyond these values is not feasible.

Activation of T4 as a Function of the Length of Exposure to L-*Tryptophan*

Plaque count method. T4 (5/10/45) was diluted 1:50,000 in 200 ml of F medium at 26 C and placed in a Waring-type blender. One-tenth ml from the suspension in the blender was then added to 1.9 ml of F medium that contained 9×10^9 B. At time t = 0, 0.4 mg of L-tryptophan dissolved in 4 ml of F were added to the contents of the blender, and the motor was turned on for 2 seconds to mix the contents. Then at intervals 0.1-ml samples were removed from the blender and added with stirring to 1.9-ml samples of the bacterial suspen-

sions, the time of addition being carefully noted. After 5 minutes had been allowed for adsorption, the B-T4 mixtures were assayed for plaque-forming particles on F and on N agar. The results presented in figure 7 show that the virus rapidly gained activity with the length of time it was exposed to L-tryptophan.

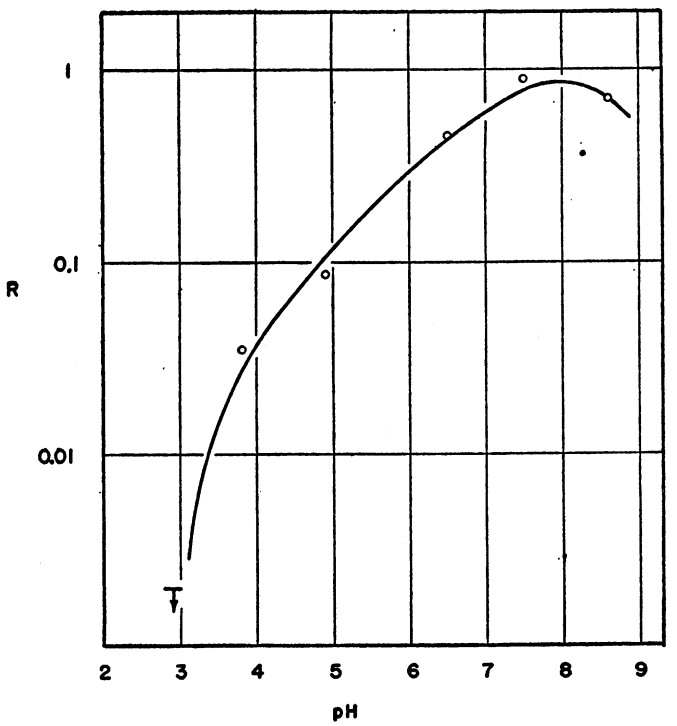

FIG. 6. THE ACTIVATION OF T4 BY 20 μG L-TRYPTOPHAN PER ML AT VARIOUS pH'S

The results were expressed as the apparent ratio R between the active to inactive virus and the values plotted above on a logarithmic scale versus the pH. The value R at pH 2.9 was determined to be less than 0.002.

The Deactivation of Tryptophan-activated T4 by Dilution in F Medium

The results presented so far indicate that the virus T4 is activated by L-tryptophan at concentrations above 0.2 μg per ml and that the extent of activation depends on the tryptophan concentration, the temperature, and the pH. A number of observations suggested that this activation is reversible. For example, T4 grown on B in nutrient medium is rapidly adsorbed onto host cells added to the suspension. However, if such a suspension is diluted 1:1,000,000 in F medium and then added to B, only 0.1 per cent of the particles will be adsorbed or form plaques on F agar. The virus must then have lost its activation on dilution in F medium. This rate of deactivation of tryptophan-activated T4 was followed in the following experiment:

One-half cubic centimeter of T4 (5/10/45) was added to 9.5 ml of F medium

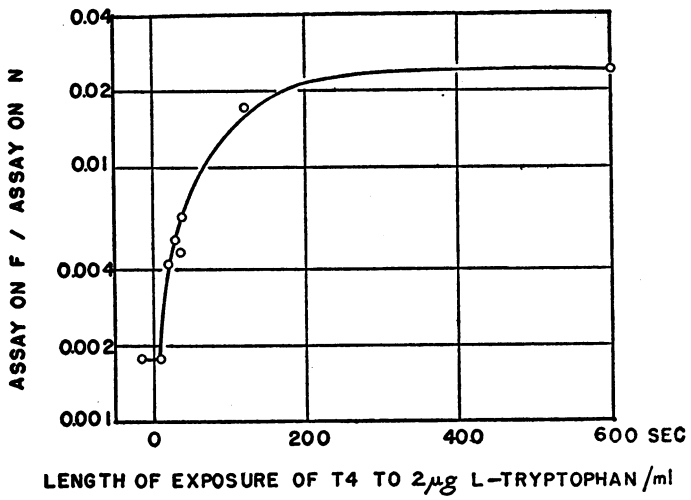

FIG. 7. THE ACTIVATION OF T4 (5/10/45) IN F MEDIUM AT 26 C AS A FUNCTION OF THE LENGTH OF EXPOSURE TO 2 μG L-TRYPTOPHAN PER ML

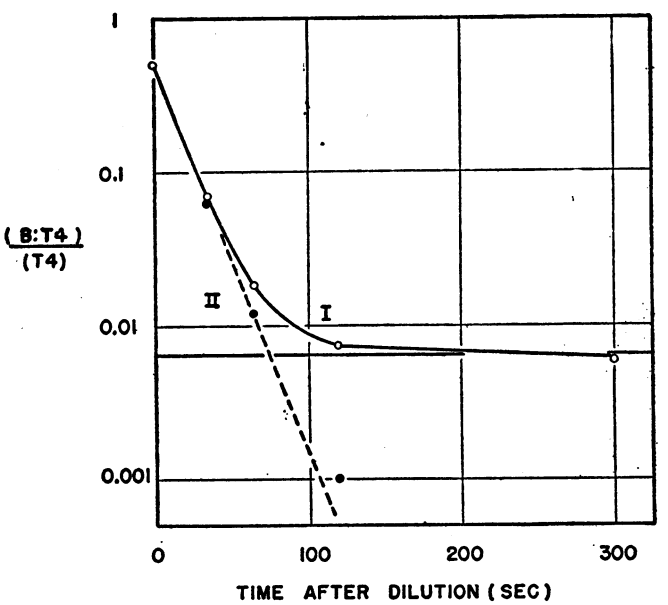

FIG. 8. THE LOSS OF ACTIVITY OF T4 ACTIVATED BY 20 μG L-TRYPTOPHAN PER ML AFTER DILUTION 1:100 IN F MEDIUM

In curve I the value of (B:T4) is seen to drop rapidly and approach the value for virus that had not been exposed to added L-tryptophan. When the latter value is subtracted from the values observed at different times the fractions plotted in curve II were obtained.

containing 20 μg L-tryptophan per ml and allowed to stand about 30 minutes at room temperature to activate the virus. At time t = 0, 0.1 ml of this suspension was added to 10 ml of F medium and stirred rapidly. Since the concentration

of tryptophan was only 0.2 μg per ml in this suspension, the virus might have been expected to lose activity with time. After various time intervals 0.1-ml samples of the diluted suspension were added to 10-ml samples of a suspension of B in F medium. This permitted the virus that had retained its activity to infect host cells to form B:T4, which, being capable of forming plaques on F agar, would provide a measure of the decreasing concentration of tryptophan-activated virus in the diluted T4-tryptophan mixture. Appropriate dilutions of the suspensions were then made and samples plated on F agar to provide plaque counts of the total number of plaque-forming particles (T4). The results, shown in figure 8, curve I, show the ratios of infected bacteria to total virus, (B:T4):(T4), plotted logarithmically against the time after dilution at which the samples were added to the bacterial suspensions. The point at zero time was obtained by adding 0.1 ml of the T4 in 20 μg L-tryptophan per ml directly to 10 ml of the bacterial suspension and assaying this suspension for infected bacteria (B:T4) and total infectious centers (T4). The horizontal line in the graph was obtained by assaying the stock T4 concentrate directly on F and N, making all dilutions in F medium. This quantity represents the fraction of the virus that could form plaques without added cofactor.

It is seen that the concentration of activated T4 in the diluted suspension of tryptophan-activated T4 drops rapidly and then levels off to approach the value for the unactivated suspension. Subtracting the value for the latter suspension from each of the points of curve I, one obtains the dotted curve II, which represents the rate of loss of activity by those T4 particles that had been activated by tryptophan. It is seen that only 2 minutes after dilution all but one 0.1 per cent of the activated virus had lost its activity. The deactivation reaction appears to be first order, but it is so rapid that it is difficult to follow at room temperature with the pipette and test tube techniques employed here. The results of more precise deactivation experiments will be reported shortly. The point to be emphasized here is that the cofactor L-tryptophan activates the virus T4 in a reversible manner.

DISCUSSION

A lower limit for the number of tryptophan molecules required for activation may be estimated from the slope of the plot of log (T4*) vs. log (tryptophan) at equilibrium. Here (T4*) represents the concentration of activated T4. Writing the reaction

$$\text{T4} + \text{n L-tryptophan} = \text{T4*}$$

the law of mass action predicts that at equilibrium

$$\log \frac{(\text{T4*})}{(\text{T4}) - (\text{T4*})} = \text{K} + \text{n} \log(\text{L-tryptophan})$$

Since the slope of the activation curve (figure 3) is approximately equal to six we judge that at least six tryptophan molecules must have been involved in the activation of T4. The observed inhomogeneity in the requirements of

the individual particles in the population (Anderson, 1948*a*) would have the effect of reducing the slope for the population.

The activation of T4 by 20 μg L-tryptophan per ml as a function of temperature is interesting; like many biological phenomena it displays a maximum at about 35 C. In enzyme systems it is usual to account for activity curves of this sort on the assumption that the reaction rate of the "native" form increases with temperature but that this form is in equilibrium with a nonreactive "denatured" high temperature form whose accumulation at the expense of the native form results in an eventual reduction in rate as the temperature is increased. The explanation of the present results may involve a similar mechanism in which the "receptiveness" of the virus for cofactor is shifted with temperature.

The inactivity of D-tryptophan as compared to L-tryptophan (Anderson, 1946*a*) suggests a specificity in cofactor phenomena comparable to that observed in enzyme systems. It would seem from the results reported here that, like substrate molecules reversibly bound to enzymes but not reacting until the combination makes liaison with coenzyme or a source of energy, so the cofactor molecules are reversibly bound in the virus structure and do not react chemically until contact with host constituents is made. Rather than a simple, static fitting together of sterically complementary structures of the virus and host, it now seems likely that the adsorption of virus on its host is a dynamic, enzymelike process. Adsorption may involve enzymatic syntheses (of peptides?) coupled with degradations of the hosts' surface elements.

ACKNOWLEDGMENT

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SUMMARY

Experiments were performed in which samples of the bacterial virus T4 were exposed to a cofactor for adsorption, L-tryptophan, under various conditions. The samples were then mixed with suspensions of the host, *Escherichia coli* B under identical conditions. It was found that the fractions of virus adsorbed varied with the conditions under which the virus had been exposed to its cofactor.

The percentage activation varied with the following factors: (a) with tryptophan concentration, it was negligible at 0.1 μg per ml and rose sharply to high values at 2 μg per ml; (b) with temperature, it was maximum at 35 C and fell to low values at 0 C and 60 C; (c) with pH, it was maximum near pH 8; and (d) with the length of exposure (equilibrium with 2 μg per ml was practically complete in 2 minutes at 26 C).

When incubated with tryptophan and then diluted in synthetic medium, the virus rapidly lost its ability to be adsorbed on its host. Upon re-exposure to an adequate concentration of a cofactor it regained its ability to be adsorbed.

It is concluded that the cofactor for adsorption, L-tryptophan, activated the bacterial virus T4 in a reversible manner.

Possible functions of virus cofactors in the adsorption mechanism are discussed.

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