

## THE MECHANISM OF VIRUS ATTACHMENT TO HOST CELLS

### I. THE ROLE OF IONS IN THE PRIMARY REACTION\*

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The essential nature of the reaction by which a virus unites with its host cell as the first step of its invasive metabolic cycle has remained obscure, despite several important discoveries in this field. The parasitism of *Escherichia coli* B by bacteriophage is particularly convenient for investigation of this mechanism, since the precise measurements which this system affords make possible quantitative study of the kinetics of virus-cell interaction. It has been previously demonstrated that the rate of adsorption of the phage particle on the host cell is directly proportional to the concentrations of both virus and bacteria; and that, for certain viruses, at least, the reaction in nutrient broth at 37°C. is so rapid as to make a relatively large proportion of the collisions between the phage and host cell effective in promoting attachment (1, 2). The reaction is highly specific, since mutant forms of susceptible cells can be produced which are immune to a given virus, though still susceptible to attack by the other strains which parasitize the original host, and also by mutant progeny of the original virus. The T system of *E. coli* bacteriophages consists of seven different phage types, characterized on the basis of the existence of specific resistant host mutants, and in some cases by differentiating morphological and antigenic properties as well (3). The adsorption on bacterial cells of some phages has occasionally been found to be accelerated by various cations, such as Na<sup>+</sup> (4, 5), although the conditions governing these requirements have rarely been precisely defined. Finally, a few deficient strains of phage have been isolated which cannot attach to host cells unless they have first reacted with an organic cofactor, particularly *l*-tryptophane (6).

In the present study, the rate of adsorption of various bacteriophages of the T system to cells of *E. coli* B, and to inorganic substrates, has been quantitatively measured under various conditions. The results of these experiments yield evidence that the initial binding of the virus to the host cell is an electrostatic one, determined by the presence of an appropriate configuration of ionic charges on the two bodies.

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### Methods

The methods employed for the preparation and storage of phage stocks, and the plaque-count technique for titrating virus activity have already been described (7). In the present experiments, young cultures of *E. coli* B, grown at 37°C. for 2½ hours in aerated nutrient broth (Difco) were employed. Such suspensions, which contained approximately 10<sup>8</sup> bacteria per cm.<sup>3</sup> were centrifuged, washed in distilled water, and, after a second centrifugation, resuspended in a freshly prepared solution which served as the adsorption medium. It was necessary to use doubly distilled water for all solutions and to clean glassware with scrupulous care, since even trace amounts of impurities were often able to affect the experimental results. The rate of virus adsorption on cells of *E. coli* B was measured by adding 0.10 cc. aliquots of the virus, diluted approximately ten thousandfold in phosphate buffer or in the medium under test, to thermostated suspensions of cells in 0.9 cc. of the test medium. The cell concentration employed was always several thousandfold greater than that of the virus, so that a pseudomonomolecular reaction resulted. The suspension was rapidly mixed, and 0.1 cc. samples were removed at various times, and immediately added to tubes containing 0.9 cc. of broth in an icebath, where the resulting dilution of the virus-cell mixture, plus the low temperature, effectively stopped all further interaction. These tubes were titred for their total virus content, then centrifuged in the cold, and aliquots of the cell-free supernatant carefully withdrawn and titred. From these two sets of plaque counts, the fraction of phage particles remaining unattached to cells was obtained as a function of time. All the experiments described were carried out at 37°C., unless otherwise indicated. Fig. 1 illustrates the results of a typical test. The adsorption constant, (defined as  $k$  in the equation  $-\frac{dP}{dt} = kPB$  where  $P$  = concentration of virus remaining unattached to cells at the time  $t$ , in the presence of a bacterial concentration  $B$ ) is readily computed from the slope of such a curve. Reproducible values of  $k$  were obtained for any bacterial concentrations up to 10<sup>9</sup>/cc. Beyond this density, the value of  $k$  tended to drop, possibly because of mutual interference by the cells in such a dense suspension.

### EXPERIMENTAL RESULTS.

1. *Virus-Cell Attachment in Nutrient Broth.*—The velocity of interaction of *E. coli* B with several bacteriophages was measured in nutrient broth at 37°C. The results are summarized in Table I, and a typical curve shown in Fig. 1.

TABLE I  
*Measurement of Velocity Constants for Attachment of Four Viruses to E. coli B in Media Indicated, at 37°C.*

Bacteriophage	Medium	$k$ = adsorption constant ( <i>cm.<sup>3</sup> min.<sup>-1</sup></i> )
T1	Nutrient broth	$310 \times 10^{-11}$
T3	“ “	$300 \times 10^{-11}$
T2	“ “	0*
T4	“ “	0*
T2	“ “ + .1 M KCl	$210 \times 10^{-11}$
T4	“ “ + .1 M NaCl	$310 \times 10^{-11}$

\* Indicates no measurable reaction.

In plain broth, phages T1 and T3 exhibited the extremely rapid adsorption rate ( $k$  between  $200$  and  $400 \times 10^{-11}$  cm.<sup>3</sup>/min.) which has been shown to be equivalent to a collision efficiency in the neighborhood of 100 per cent for such systems (1, 2). Phages T2 and T4, however, exhibited no reaction whatever, until NaCl or KCl was added to the medium (5). When salt concentrations of the magnitude indicated in the last two lines of Table I were attained, these viruses also exhibited the same very high rate of reaction. Throughout the course of this study, no attachment velocity greater than  $k = 450 \times 10^{-11}$  cm.<sup>3</sup> min.<sup>-1</sup> was ever observed in any experiment, despite the wide range of conditions employed, a fact which lends support to the theoretical analysis which predicts that this region of values should be the maximum attainable rate in aqueous solution for bodies with the geometrical relations which characterize these phages and their host (1, 2).

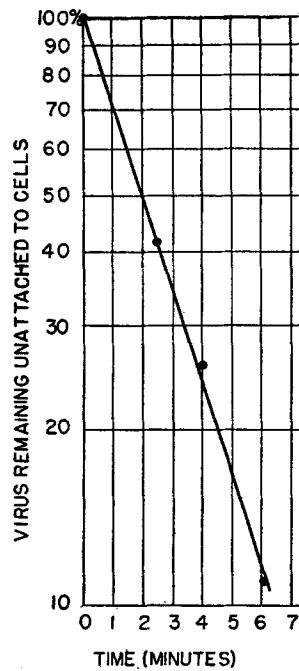


FIG. 1

FIG. 1. Typical curve showing the linearly logarithmic relation obtained when the concentration of free T1 phage in a tube also containing host cells is plotted against the time. The initial virus concentration in this experiment was  $4 \times 10^4$  particles/cm.<sup>3</sup>. The cell concentration was  $2.57 \times 10^8$ /cm.<sup>3</sup> so that the adsorption velocity constant,

$$k = \frac{2.3}{6 \times 2.57 \times 10^8} \log \frac{100}{12.1} = 130. \times 10^{-11} \text{ cm.}^3 \text{ min.}^{-1}.$$

FIG. 2. The effect of (A) NaCl and (B) CaCl<sub>2</sub> on the velocity of attachment of T1 bacteriophage to host cells in buffered solutions of the pure salts. Curves essentially similar to (A) were obtained with KCl, LiCl, and NH<sub>4</sub>Cl, while MgCl<sub>2</sub>, BaCl<sub>2</sub>, and MnCl<sub>2</sub> produced behavior like that of (B).

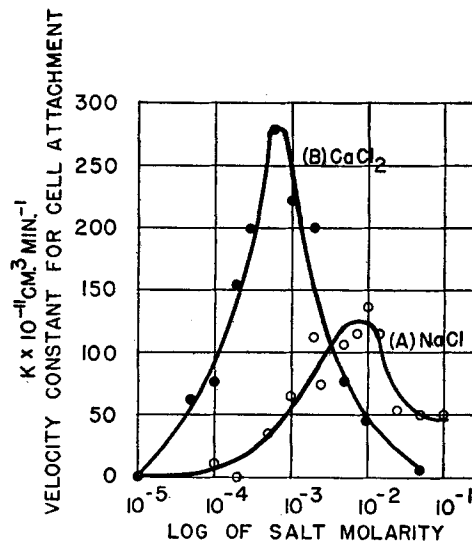


FIG. 2

The velocity of this reaction can be more readily appreciated when one considers the extreme dilutions employed in the experiments, in order to slow down the interaction to the point where it could be studied. Thus the concentration of cells employed was usually between  $10^7$  and  $10^8$ /cc.; while the bacteriophage concentration was  $10^{-12}$  gm./cm.<sup>3</sup> or a virus molarity of  $10^{-17}$ . The fact that the reaction between cell and virus proceeds more than 90 per cent to completion within 5 to 10 minutes at such concentrations of the reactants makes clear the magnitude of this attachment rate.

2. *Rate of Reaction in Chemically Defined Media.*—Analysis of the reaction kinetics of virus-cell attachment in media of known chemical composition was carried out, using T1 bacteriophage in particular, as a representative member of this virus group. No measurable attachment to *E. coli* B occurs when the two are suspended in distilled water, or in  $10^{-4}$  M phosphate buffer. In most of the experiments which follow,  $10^{-4}$  M phosphate buffer at pH 6.8 was used as the basic medium to which substances under test were added.

(a) *Effect of Univalent Ions.*—The addition of NaCl markedly raises the adsorption rate of T1 virus. When a concentration of approximately 0.005 M NaCl is attained a reaction velocity is reached which is almost half that obtained in nutrient broth. However, if still greater concentrations of NaCl are employed, the reaction velocity becomes depressed, ultimately again reaching fairly low values at salt concentrations greater than 0.05 M. These experiments are summarized in Fig. 2 (A curve) in which the adsorption velocity constant is plotted as a function of the NaCl concentration of the medium.

Almost identical curves are obtained if the NaCl is replaced by LiCl, KCl, or NH<sub>4</sub>Cl in these experiments. Furthermore, the depressing action of an excess of NaCl is also exerted in nutrient broth. The addition to broth of 0.1 M NaCl, for example, markedly diminishes the rate of the attachment reaction to a value comparable to that obtained in synthetic medium at the same salt level as shown in Figure 2.

(b) *Effect of Divalent Ions: Ca, Mg, Ba, Mn.*—As Fig. 2 also demonstrates, CaCl<sub>2</sub> displays a general pattern similar to that of NaCl in influencing the adsorption rate, except that the entire curve is displaced to the left by a factor of about 10 in concentration, and a higher attachment rate is obtained. Thus, much lower concentrations of CaCl<sub>2</sub> are required, both to achieve the maximal adsorption rate, and later to inhibit this reaction, and at optimum concentration of Ca<sup>++</sup>, the velocity constant is as high as that observed in nutrient broth. The difference in behavior of NaCl and CaCl<sub>2</sub> is obviously due to the cations, since the anion is the same. Moreover, the nitrate and sulfate behave exactly like the chloride. The small amount of phosphate buffer present in these experiments does not appreciably affect the adsorption velocity, as shown by the fact that solutions of pure CaCl<sub>2</sub> produce effects identical with those of the buffered solution. As in the case of the univalent cations, the effect of Ca<sup>++</sup> ion was shown to have a charge specificity, but not to be otherwise unique, as

curves essentially the same as Fig. 2 (*B* curve) were obtained when  $\text{CaCl}_2$  was replaced by  $\text{MgCl}_2$ ,  $\text{BaCl}_2$ , or  $\text{MnCl}_2$ .

(*c*) *Trivalent Ions.*—

It was not possible to observe the effect of trivalent cations like  $\text{Fe}^{3+}$ ,  $\text{Cr}^{3+}$ , and  $\text{Al}^{3+}$  on this reaction, since salts of these ions, even in very high dilution, produced rapid and irreversible inactivation, both of the virus and the host cell.

Since the extremely high reaction rate of nutrient broth suspensions can be duplicated by solutions of pure salts, and since the non-ionized constituents of broth fail to prevent the depression in reaction rate caused by an excessive ion concentration, it may be concluded that the rate of cell invasion by T1 virus is controlled completely by the ionic constituents of the medium. Moreover, for a large number of such ions the only specificity exhibited is related to the total charge, rather than to the chemical species of the ion. These considerations indicate that electrostatic forces play an important role in this attachment reaction. A search was directed, therefore, to determine whether, indeed, any other process like an enzyme transformation is also involved, or whether the union of the virus to the cell is, in its first stages, at least, completely an ionic interaction, comparable perhaps, to the attachment of an organic molecule with acid or basic groups, to an ion-exchange resin.

3. *Rate of Activation of the Adsorption System by Metallic Cations.*—Experiments were carried out to measure how quickly inorganic ions can convert a non-reacting mixture of virus and cells into a rapidly reacting one, since the demonstration of an appreciable lag period might be evidence for the existence of slower transformations, possibly enzymic in nature, which must precede the actual attachment process. *E. coli* B and T1 bacteriophage were mixed in distilled water at 37°C. The mixture was sampled after various time intervals and the extent of adsorption at each moment determined. At the end of 3 minutes,  $\text{MgCl}_2$  was rapidly added in an amount sufficient to make its final concentration in the adsorption tube  $10^{-3}$  M. A sample was removed immediately afterward, and others at subsequent intervals. The resulting curves, a sample of which is shown in Fig. 3, indicate that the rate of reaction of  $\text{Mg}^{++}$  with the system is so rapid as to be beyond the resolving time of the experimental procedure. One can conclude that the half-life of activation of the system by  $\text{Mg}^{++}$  is much shorter than 20 seconds.

4. *Temperature Coefficient of Virus Attachment on Cells.*—The effect of temperature on the rate of adsorption of T1 on *E. coli* B was studied, both in broth and in solutions containing only  $\text{MgCl}_2$  and buffer. The curves obtained in these two media were practically identical, (Fig. 4) and exhibited a maximum adsorption rate in the neighborhood of 37°C., which falls off with increasing or decreasing temperature. The identity of the results in broth and in  $\text{MgCl}_2$

solution indicates that no organic cofactor dependence arises, even at a temperature as low as 5°C. The significance of the shape of this temperature curve will be discussed in a subsequent paper (12).

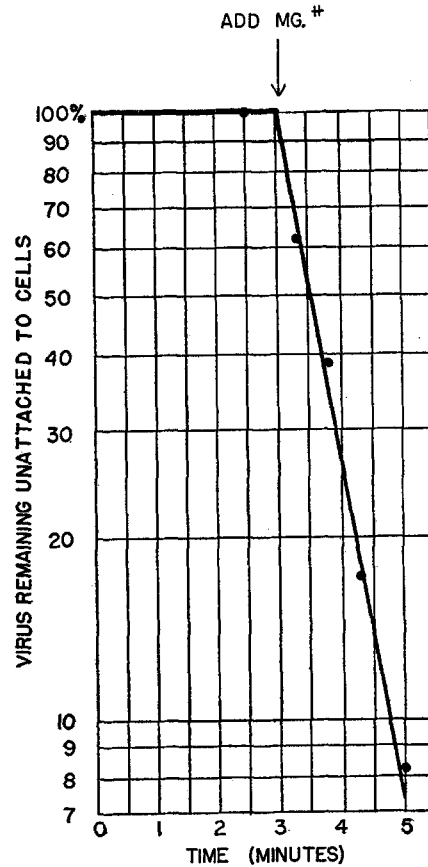


FIG. 3. Experiment showing absence of any lag period when an inert mixture of T1 phage and host cells in distilled water is activated by the addition of  $MgCl_2$ . Virus attachment to cells starts immediately at the maximum possible rate. ( $K = 230 \times 10^{-11} \text{ cm.}^3 \text{ min.}^{-1}$ ).

5. *Attachment of Bacteriophage to Inorganic Surfaces.*—The preceding experiments lend themselves to a simple interpretation, namely that the union of a virus to its host cell consists, for the first step at least, of an electrostatic attachment between points of opposite polarity distributed in complementary fashion over the two surfaces. The role of the inorganic ions may be visualized as simple addition to specific sites on the two bodies, thereby furnishing the charge distribution necessary for virus-cell attachment. Fig. 2 indicates that

in low concentration ranges the addition of cations increases the reaction velocity, while higher concentrations inhibit the process. This dual role of  $\text{Ca}^{++}$ , for example, could indicate that it can become bound to two different kinds of sites in this system—attachment at the “first kind” of site aiding the course of cell invasion, while attachment at the “second” blocks the process.

In distilled water, then, all the sites on both surfaces at which ion attachment is possible, will be vacant. Hence no reaction between virus and cell takes place, because the electrostatic pattern necessary for attachment is not fulfilled. When a salt like  $\text{CaCl}_2$  is added to the medium in a concentration of  $10^{-3}$  M, the  $\text{Ca}^{++}$  ions become bound only to negative sites of the “first kind” (which may be distributed on one or both of the reacting bodies) with the

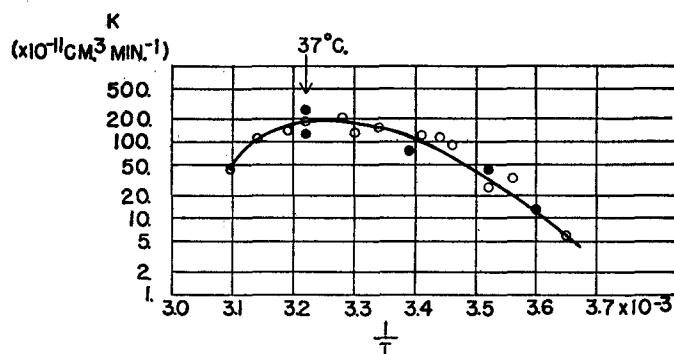


FIG. 4. Change of velocity constant for virus-cell attachment with temperature. Solid circles indicate experiments in phosphate buffer +  $10^{-3}$  M  $\text{MgCl}_2$ . Open circles are nutrient broth. A significant rate of reaction is observed even at temperatures as low as  $1.0^\circ\text{C}$ . (Abscissa is reciprocal of the Absolute temperature.)

result that the two surfaces present patterns which are geometrically and electrostatically complementary. Virus and cell will then adhere on contact. However, if an excess of  $\text{CaCl}_2$  is added to the solution, the  $\text{Ca}^{++}$  ions will begin to attach to sites of the “second kind” and so interfere with union of virus and bacterium. A highly diagrammatic scheme illustrating how the system might operate is shown in Fig. 5. The fact that a similar series of effects can also be achieved by univalent cations like  $\text{Na}^+$ , provided that a much higher concentration is employed, is readily understood as evidence that the binding energy of  $\text{Na}^+$  to the sites which require a positive charge for fulfillment of the necessary pattern, is smaller than that of  $\text{Ca}^{++}$ . Hence, a greater concentration of  $\text{Na}^+$  in the external medium is required to achieve the same equilibrium charge distribution of attached ions on negative sites of virus and cell surfaces. Such a difference would be expected, in view of the usual differences of bond strengths for singly and doubly charged ions to most negatively charged organic radicals.

If this formulation is valid, it should be possible to replace the host bacterial cells by a polar inorganic surface with a sufficiently great variety of electrostatic configurations so that patterns complementary to those of specific virus-

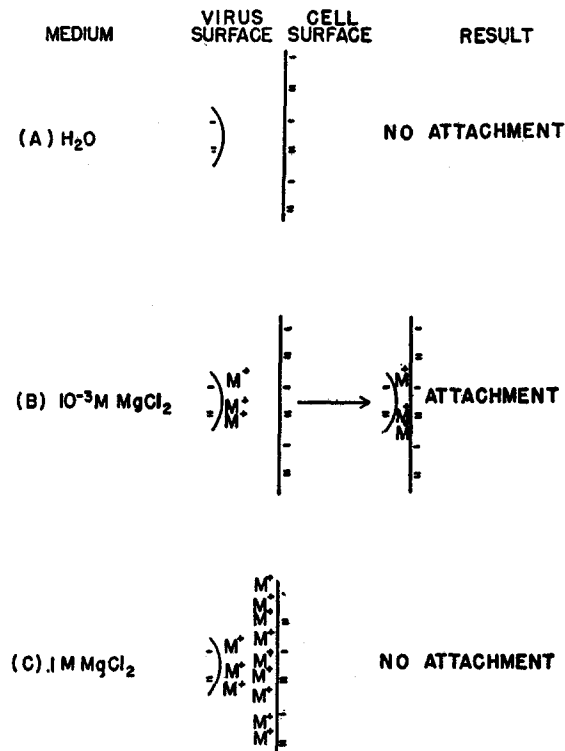


FIG. 5. Highly schematic diagram showing one possible arrangement which would operate according to the proposed hypothesis. The essential assumption is that two kinds of sites for cation attachment exist on the surfaces of the cell and virus. Attachment of cations to sites of the first kind is necessary for virus adsorption on cells, while attachment of cations to sites of the second kind blocks the process. The cation-binding energy for all the ions studied here is greater for sites of the first kind than for those of the second.

For the sake of diagrammatic simplicity, the sites of the first kind have been pictured as situated only on the virus, and those of the second kind only on the cell. Further experiments are being carried out to clarify the actual distribution of these two kinds of sites on the two surfaces (12). In (A) and (C), the configurations on cell and virus will repel each other. In (B), an electrostatically complementary configuration has been attained permitting union.

cation complexes will occur. In that case, it should be possible to duplicate many of the phenomena of virus-cell attachment. In 1940 Delbrück reported experiments on a strain of bacteriophage that could be made to attach reversibly to a Jena glass filter, a procedure he employed to concentrate phage suspensions



(8). His results, however, were not always reproducible when applied to other viruses (9). It was decided to reinvestigate this phenomenon since, if this adsorption could be shown to be basically similar to the interaction of phage with host cells, conclusions about the electrostatic nature of the biological reaction would be materially strengthened.

Experiments revealed that T1 bacteriophage could readily be adsorbed onto glass filters. This finding by itself does not furnish a very searching test of the proposed theory. A more critical experiment was devised using a tryptophane-requiring mutant of T4 bacteriophage. This phage, originally isolated by Anderson (6) can only attach to cells of *E. coli* B if it has first reacted with *l*-tryptophane in a concentration of approximately 10 to 20  $\gamma$ /cc. It has been demonstrated that the activating effect of tryptophane is confined to the virus, no effect being exerted on the cell itself. Now, if the attachment of a virus to an inorganic polar surface of this kind is simply a non-specific type of adsorption between the glass and the multifold polar and non-polar chemical groupings present on the nucleoprotein virus molecule, tryptophane should not be expected to influence this reaction. But, if the union of the phage to the filter involves the same chemical groupings which determine virus-cell interaction, any biochemical specificities involved in the latter case should also be exhibited in the former. Experiments were performed comparing the effect of filtering this mutant virus when suspended (a) in phosphate buffer plus  $2 \times 10^{-4}$  M  $MgCl_2$ , and (b) in the same medium to which tryptophane had been added. Such experiments revealed that this particular virus mutant is strongly adsorbed to the glass filter only in the presence of tryptophane. A typical protocol is presented in Table II.

From this T4 virus stock (which had been prepared from a single plaque) a mutant requiring no tryptophane for cell invasion was isolated by plating a massive inoculum of the virus with *E. coli* B on an agar plate containing a synthetic medium lacking tryptophane<sup>1</sup> but capable of supporting growth of the bacterial host. A small number of plaques resulted, one of which was isolated and used to prepare a new stock. This strain of T4 phage did not require tryptophane for cell invasion. Experiment showed that it is also adsorbed on glass filters with high efficiency in the *absence* of tryptophane. A representative experiment is shown in Table III, in which the action of sufficient and deficient mutants is compared.

That this removal of phage by glass filters represents a true attachment of the virus to the filter, rather than some destructive action accompanying the filtration, was demonstrated by the fact that such virus could be reelected by the use of an appropriate medium. Nutrient broth plus 0.5 per cent NaCl

<sup>1</sup> The composition of this synthetic medium is as follows: glucose, 4.0 gm.;  $(NH_4)_2SO_4$ , 1.0 gm.;  $KH_2PO_4$ , 0.75 gm.;  $Na_2HPO_4$ , 1.75 gm.;  $MgCl_2 \cdot 6H_2O$ , 0.20 gm.;  $CaCl_2 \cdot 2H_2O$ , 0.15 gm.; bacto-agar 14 gm.;  $H_2O$  to 1 liter.

proved to be an excellent elution fluid for all the phages tested, presumably because the enormous excess of protein in the broth is able by mass action to replace virus molecules from the active attachment sites on the filter. In the experiment described in Table III, for example, more than 50 per cent of the virus activity removed from the original suspension in trials (a) and (c) was reelected when 5.0 cc. quantities of broth were passed twice through each filter.

TABLE II  
*Demonstration of Cofactor Action of Tryptophane for Attachment of Deficient T4 Bacteriophage to Glass Filters*

Concentration of tryptophane in suspending medium	T4 virus titre before filtration	Virus titre in filtrate	Virus removed by filter
0	$3.93 \times 10^6/\text{cm.}^3$	$3.60 \times 10^6$ cc.	8*
20 $\gamma$ /cc.	$3.98 \times 10^6/\text{cm.}^3$	$0.33 \times 10^6$ cc.	90.5

Two 50 cc. samples of a broth lysate of a tryptophane-requiring T4 mutant† were diluted  $1:10^4$  in 0.014 M phosphate buffer +  $2 \times 10^{-4}$  M  $\text{MgCl}_2$ . To one sample, *l*-tryptophane was added in a concentration of 20  $\gamma$ /ml. Each sample was filtered twice through a Corning fritted glass bacterial filter (catalog No. 33990). The virus titre of each suspension was determined before and after filtration.

\* Within experimental error.

† Kindly supplied by Dr. Max Dellbrück.

TABLE III  
*Demonstration that Tryptophane Is Required for Attachment to Glass Filters Only by the Virus Mutant Which Also Requires Tryptophane for Invasion of Cells*

Mutant strain of T4	Concentration of tryptophane in suspending medium	Virus titre before filtration	Virus titre of filtrate
(a) Sufficient form	0	$2.45 \times 10^6$	$0.14 \times 10^6$
(b) Tryptophane-deficient form	0	$3.39 \times 10^6$	$3.20 \times 10^6$
(c) " "	10 $\gamma$ /cc.	$2.01 \times 10^6$	$0.65 \times 10^6$

#### 6. Temperature and Salt Effects in Adsorption of T1 Bacteriophage on Filters.

—Since the attachment of bacteriophage to the highly polar surface of a glass filter can partake of the biochemical specificity of phage invasion of host cells, study of the kinetics of the former system should throw light on the mechanism of the latter. The effect of temperature was studied first. A preliminary investigation showed that  $10^{-3}$  M  $\text{MgCl}_2$  solution is an excellent medium for adsorption of T1 on the glass filters. To test the effect of temperature on this action, parallel filtrations were carried out at temperatures of 37° and 3.0°

C., in the latter case the entire procedure taking place in a cold room. As Table IV illustrates, practically complete adsorption occurred at both temperatures.

Since effective adsorption is obtained at low temperatures, study of the salt effects was carried out at a temperature near 0°C., to reduce still further the virus inactivation which occurs at low ionic strengths (10). A slight excess of

TABLE IV  
*Demonstration of Completeness of Adsorption of T1 Virus on Glass Filters at Both High and Low Temperatures*

Filtration temperature	T1 titre before filtration	T1 titre of filtrate
37°	$1.53 \times 10^8/\text{cc.}$	$0.001 \times 10^8$
3.0°	$1.73 \times 10^8/\text{cc.}$	$0.001 \times 10^8$

TABLE V  
*The Effect of Mono- and Divalent Salts on the Adsorption Efficiency of T1 Bacteriophage on Glass Filters*

Filtration medium	Ratio: $\frac{\text{titre of solution after filtration}}{\text{titre before filtration}}$ <i>per cent</i>
(A) H <sub>2</sub> O	38.0*
(B) 10 <sup>-4</sup> M CaCl <sub>2</sub> 10 <sup>-3</sup> M CaCl <sub>2</sub>	17.0 less than 0.1
(C) 10 <sup>-3</sup> M MgCl <sub>2</sub>	" " 0.1
(D) 10 <sup>-3</sup> M NaCl 10 <sup>-2</sup> M NaCl	8.5 less than 0.50

\* This value actually represents inactivation by the distilled water, rather than even a small amount of adsorption, as shown by the fact that no virus could be eluted when nutrient broth was poured through the filter after the water filtration, whereas, in the case of the other media listed in the table, a subsequent washing with broth resulted in recovery of almost all the virus which had disappeared from the original suspension.

MgCl<sub>2</sub> was also added to the collecting tubes, so that immediately after its passage through the filter, the virus was restored to an ionic atmosphere in which it is stable. Even with these precautions, appreciable inactivation occurred in the case in which distilled water or 10<sup>-3</sup> M NaCl was used as the suspending medium. Despite this disturbing effect, it was possible to demonstrate markedly parallel action between the adsorption of T1 on glass filters and its attachment to host cells. The experiments of Table V indicate (a) little or no

virus adsorption on the glass occurs in distilled water; (b)  $\text{CaCl}_2$  produces some effect in a concentration of  $10^{-4}$  M, but does not achieve optimum action until a concentration of  $10^{-3}$  M is attained; (c) other divalent salts like  $\text{MgCl}_2$  are effective in the same concentration as  $\text{CaCl}_2$ ; (d) if monovalent salts are employed, a tenfold greater concentration is required for maximum effectiveness,  $\text{NaCl}$  being only moderately efficient at  $10^{-3}$  M, but an excellent adsorption medium in a concentration of  $10^{-2}$  M.

In contrast to these noteworthy similarities in the action of a glass filter and cells of *E. coli* B on T1 virus in dilute salt solutions, more concentrated solutions revealed an important difference in these two systems. Thus, whereas increase in the concentration of  $\text{Ca}^{++}$  ultimately results in depression of the virus attachment to cells, (Fig. 2), no such action is observed in the case of the filters. Even 0.5 M  $\text{CaCl}_2$  promoted rapid attachment of more than 99 per

TABLE VI  
*Per cent Reelution by Various Media of Virus Deposited on a Glass Filter*

Elution fluid	Eluted <i>per cent</i>
$10^{-3}$ M $\text{CaCl}_2$	0 (<0.03)
$\text{H}_2\text{O}$	60
Nutrient Broth + 1/2 per cent $\text{NaCl}$	70

5.0 cc. quantities of each fluid were passed once through a filter on which virus had been deposited by previous filtration in  $10^{-3}$  M  $\text{CaCl}_2$  or  $10^{-3}$  M  $\text{MgCl}_2$  solution.

cent of the virus to the glass surface, almost all of which was subsequently recoverable by broth elution. Experiments at still higher concentrations of  $\text{CaCl}_2$  were not feasible because of the onset of permanent inactivation of the phage in such solutions.

7. *Reversibility of Virus Attachment to Filters and to Cells.*—The action of nutrient broth in causing reelution of bacteriophage attached to glass filters has already been described. In addition, however, it was found that virus adsorption on glass filters is readily reversible through control of the ionic constituents alone. Thus, a medium which promotes good attachment of T1 to the glass filter will not produce any appreciable elution of phage previously deposited, whereas solutions from which adsorption does not take place are excellent eluting agents. As shown in Table VI,  $10^{-3}$  M  $\text{CaCl}_2$  will not liberate free phage which has been deposited on a glass filter, while a single passage of distilled water through the filter liberates most of the adsorbed virus. This kind of reversibility is exactly that expected for an ionic attachment of molecules with acid or basic groups to a complex silicate surface like that of the glass filter. The clarification of these relationships for a variety of viruses should make possible rapid and simple means for their purification and concentration.

The theory that the primary attachment of a virus to its host cell is essentially similar to its adsorption on an ion-exchange surface would demand that virus-cell attachment could also be reversed. Previous studies of such infected cells had failed to demonstrate any appreciable detachment of such virus with the result that the union of bacteriophage and its host cell has come to be regarded as irreversible (1, 2). In these earlier studies, however, the attempt to reverse the reaction was carried out in the same medium in which attachment had

TABLE VII

*Demonstration of the Reversibility of Virus Attachment to Host Cells; Removal of the Virus from Infected Cells Occurs Only in Media Where the Forward Reaction Does Not Proceed.*

*Procedure.*—It was necessary to employ T2 virus for these experiments, because distilled water, used as the reelution medium, causes extensive inactivation of T1.

In a tube at 37°,  $5 \times 10^8$  T2 virus particles were added to  $5 \times 10^8$  cells of *E. coli* B in 1 cc. of  $10^{-3}$  M phosphate buffer + 0.02 M NaCl. 3 minutes was allowed for attachment to cells, after which the tube was chilled suddenly in an ice bath, titred, and centrifuged in the cold. Virus titres of the total suspension and of the supernatant, respectively, revealed that 99.5 per cent of the virus had become attached to cells. The supernatant, containing any free virus, was then discarded as completely as possible, and the remaining cells, both infected and non-infected resuspended in 0.5 cc. of distilled H<sub>2</sub>O. 0.1 cc. of this suspension was then added to (A) 1.9 cc. of distilled water, and to two media promoting very rapid cell invasion, one synthetic, (B), and the other nutrient broth, (C). After 5 minutes at 3.0°C., all the tubes were centrifuged and the virus titres of each total suspension, and of each supernatant determined. A second reelution procedure in each medium was also carried out.

Original No. of virus-infected cells in each tube	No. of free virus particles liberated into supernatant after two 5-minute washings at 0°C. with:		
	(A) H <sub>2</sub> O	(B) Synthetic medium promoting very rapid cell-attachment  ( $10^{-3}$ M Phosphate + 0.1 M NaCl + $10^{-4}$ M Mg <sup>++</sup> )	(C) Nutrient broth + .1 M NaCl  (Rapid attachment medium)
$4.98 \times 10^4$	$3.67 \times 10^4$	$0.16 \times 10^4$	$0.08 \times 10^4$

occurred, usually nutrient broth. In the light of the present considerations, the failure to observe reversal under these conditions is not unexpected. A series of experiments was performed which revealed that, whereas a medium which promotes rapid cell attachment causes practically no elution of virus from the cells, a medium like distilled water will produce extensive liberation of virus from cells which had been previously infected in a favorable environment. In a procedure closely analogous to the elution of phage from glass filters, it was possible to remove more than 70 per cent of the bacteriophage from cells to which it had become attached and to demonstrate their existence as free, active

virus particles. Table VII presents the results of a representative experiment, comparing the effects of various media on virus elution from infected cells. These relationships demonstrate that the primary step of union between a virus and its host cell is a reversible process.<sup>2</sup>

8. *Relationship between Electrostatic Bindings and Host-Virus Specificity.*—The foregoing experiments indicate that the bacteriophage-cell system can enter into a binding which partakes of at least some of the characteristics of an electrostatic bond. It is most important to determine whether this bond contains within itself the basis for the specificity of the host-virus relationship, or whether it acts simply as a non-specific device for promoting rapid and intimate contact between the invading system and the cell, so that, if the virus and the host are correctly matched, more specific bonds will then be established.

The first type of experiment designed to elucidate this problem deals with an attempt to measure whether any reaction whatever takes place when a

TABLE VIII

*Demonstration that No Binding of T1 Bacteriophage Occurs on a Specifically Resistant Cell Mutant Even in Concentrations a Thousandfold Greater Than That at Which the Wild Type Binds Virus Extensively.*

Bacteria in adsorption tube	Bacterial concentration	Total virus removed from supernatant after 10 minutes at 37°
		<i>per cent</i>
(a) <i>E. coli</i> B/1,5	$1.95 \times 10^{10}$	0
(b) <i>E. coli</i> B	$1.95 \times 10^7$	45.9

virus is brought in contact with a resistant mutant of the original host cell. If the electrostatic forces here considered are non-specific ones, serving only to promote a preliminary attachment of the virus to susceptible or resistant cells alike, it should be possible to demonstrate some interaction between a given virus and mutants of the host cell which are resistant only to this particular virus, but still sensitive to other viruses of the system. On the other hand, if these forces also determine virus specificity, one would expect that such resistant cells would have no tendency whatever to bind the virus. Experiment revealed that, in contrast to the very rapid, reversible, ion-controlled union of a virus and its specific host (Table VII), no detectable reaction occurs between a virus and a cell mutant specifically resistant to it, even at the highest cell concentration attainable. (Table VIII.)

A mutant of *E. coli* B, resistant to T1 bacteriophage, was isolated by exposing a cell suspension to a large excess of the virus on a nutrient agar plate. Colonies which appeared after

<sup>2</sup> Under certain conditions the infected cell loses its capacity to reeulte the virus. These experiments will be described later.

incubation were picked and subcultured, and tested for their resistance to plaque formation by T1 bacteriophage. A pure strain of a form resistant to phages T1 and T5, but readily attacked by all the rest, was selected and young cultures prepared by incubation in broth at 37°C. for 4 or 5 hours. Large volumes of such cells were centrifuged and concentrated several hundredfold by resuspension in very small volumes of fresh nutrient broth. To the resulting suspensions, aliquots of T1 virus were added. At intervals varying from 5 to 40 minutes the fraction of virus remaining unattached to cells was determined by titrating the virus content of the supernatant remaining after centrifugation of the cells. A typical experiment shown in Table VIII (a) reveals that even extremely concentrated suspensions of the resistant cells (designated B/1, 5) failed to bind any bacteriophage. By contrast, in line (b) it is shown that a thousandfold more dilute suspension of the wild-type host cell, prepared in exactly the same manner as the B/1, 5 cells, attached to itself approximately half of the virus particles present in the suspension.

In other experiments, concentrations of B/1, 5 cells as high as  $2.2 \times 10^{11}/\text{cm.}^3$  failed to bind any detectable amount of T1 virus, even after incubation in broth at 37°C. for 25 minutes. This failure to observe any reaction whatever between a virus and a cell specifically resistant to it, makes almost inescapable the conclusion that the ion-controlled forces are also the seat of the biological specificity in virus-cell interaction.

Another line of investigation also tends to support this conclusion. If the ion-controlled forces here described do determine virus-host specificity, one should expect to find marked differences in the pattern of ionic influence on cell-attachment rate in viruses which differ in their host specificity relationships. That is, if the electrostatic forces between virus and host cell are non-specific ones, one might expect similar influences of various ions on the attachment of the different bacteriophages to the same host cell. On the other hand, if these electrostatic bonds are characteristic for each virus type, marked differences in the ionic response of the different virus systems would be expected, since each virus must be attaching itself to different elements of the electrostatic configuration on the host-cell surface, and therefore a different ionic atmosphere might reasonably be expected, in order for each virus type to achieve the necessary patterns complementary to its own constellation of attachment points. Experiment readily revealed the existence of pronounced differences in the effects of various ions on the invasion of *E. coli* B by various members of the T system of bacteriophages. For example, under conditions which provide the maximum adsorption efficiency for T1 virus on *E. coli* B— $5 \times 10^{-4}$  M  $\text{MgCl}_2$  solution—the T2 virus is completely inert toward the same host cell. Increasing the concentration of  $\text{MgCl}_2$  to 0.01 M causes only a very slow attachment of T2 to take place ( $K = 2.4 \times 10^{-10}$  min.<sup>-1</sup>) and at higher concentrations of  $\text{Mg}^{++}$  extensive virus inactivation takes place in the adsorption tubes. On the other hand, addition of NaCl or KCl promotes very rapid reaction, and in concentrations of 0.1 M, a reaction rate of  $2.1 \times 10^{-9}$  is attained which is comparable to the optimum observed

with the T1 virus. As noted in Table I, even in nutrient broth, *E. coli* B is not appreciably attacked by T2 virus, unless 0.1 M KCl is added. By application of these facts, it has become possible to devise synthetic media such that if *E. coli* B is added to a mixture of both T1 and T2 viruses, only one or the other virus will attack the host cell to any marked extent.

Still different ionic behavior patterns have been found for the adsorption of T3 and T4 viruses to *E. coli* B, and will be described in detail elsewhere. It should be emphasized, however, that in every case investigated, the adsorption rate of each virus in its own chemically defined medium of optimum effectiveness always approached the value between 200 and  $400 \times 10^{-11}$  which is obtained in nutrient broth (Table I).

These experiments indicate that different viruses require different ionic environments in order successfully to invade a given host cell. This result is consistent with the picture that the particular configuration necessary for the attachment of each virus is attained through the binding of ions from the medium to sites on both cell and virus, to produce an electrostatic pattern which permits union. By this picture, the basis of virus-host specificity with respect to the attachment reaction would be determined by two factors: the ion-binding energies of different sites on virus and cell surfaces, determining the number of ions of each kind which becomes attached to each surface in media of various compositions; and the distribution of these sites over the two surfaces which determines the degree of complementariness between the two patterns so arising.

#### DISCUSSION

These experiments describe various aspects of the role played by inorganic ions in the reaction by which bacterial viruses become attached to their host cells. Demonstration of this relationship has suggested that the primary interaction may be largely of an ionic nature. This conception is at least able to fit together in an extremely simple picture all the observations presented here, as well as to provide an explanation for some hitherto inexplicable features of this reaction:—

1. The extremely rapid reaction rate between virus and host cell under optimum conditions demands a mechanism with practically no activation energy, such as the union of two bodies between which there exists an attractive electrostatic force.

Schlesinger and Delbrück (1) originally showed that the very high velocity of this reaction demands that almost every collision between phage particles and bacterial surface results in effective attachment. The increased refinement in measurement of the physical constants of this system since these two papers appeared have served only to confirm this conclusion. This may be demonstrated with the simplified formula of Delbrück:  $k_{\max.} = 4\pi Da$  where  $k_{\max.}$  is the maximum possible reaction



velocity constant attainable if every virus collision with any part of the bacterial surface is effective;  $D$  is the diffusivity of the phage particle; and  $a$  is the effective average radius of the bacterium. In the case of T4 bacteriophage, for example,  $D$  has been found to be  $0.80 \times 10^{-7}$  cm.<sup>2</sup> sec.<sup>-1</sup> (11), while  $a$  for the bacteria in the experiments used here is  $8 \times 10^{-5}$  cm., since microscopic measurement of a series of cells grown under the standard conditions here adopted gave values of  $1\mu \times 3\mu$  as the average bacterial dimensions.  $k_{\max}$  then is  $80 \times 10^{-12}$  cm.<sup>3</sup> sec.<sup>-1</sup> or  $500 \times 10^{-11}$  cm.<sup>3</sup> min.<sup>-1</sup> which agrees with the experimental values which lay between 200 and  $400 \times 10^{-11}$  cm.<sup>3</sup> min.<sup>-1</sup>.

This very rapid rate may be contrasted with the low collision efficiencies characteristic of systems in which reaction takes place between pairs of covalent bound atomic groupings, so that energy is required to cause the original bonds to open before new ones can be formed. Even when such systems are very effectively catalyzed, only a small proportion of the molecular collisions is sufficiently energetic to produce reaction. On the other hand, the union of charged entities like  $\text{Ag}^+$  and  $\text{Cl}^-$  ions can occur in almost every collision because of the attractive force which operates continuously even while they are several atomic diameters apart. In the case of the virus-cell combination, enzymatic reactions doubtless begin very quickly after the virus has become attached to its host, but the primary act of union is probably not an enzyme-catalyzed formation of new covalent bonds.

2. The rate of interaction of several viruses and their host cells in chemically defined media can be adjusted to any desired value between zero and the maximum theoretically possible rate, by control of the ionic constitution of the medium alone.

3. The activation by  $\text{Mg}^{++}$  of an inert mixture of virus and cells transforming the system into one which reacts at practically complete collision efficiency occurs instantaneously within the limits of present experimental technique. Thus, not only is the attachment itself extremely rapid, but also no lag period is demonstrable in the attainment of the condition of high reactivity through addition of the necessary ions. This behavior which would be unexpected in reaction mechanisms involving changes in covalency linkages, becomes readily explainable in terms of an electrostatic mechanism.

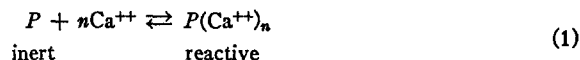
4. For a fairly large group of positive ions strongly influencing this reaction, the only specificity exhibited is associated with total ionic charge. This observation may be interpreted as evidence that the reaction requires a certain fairly gross charge distribution rather than interaction of specific chemical forces of a more subtle nature. Similar ionic relationships govern the aggregation of colloidal dispersions by electrolytes, which is primarily electrostatic in nature.

5. This hypothesis has accurately predicted that the adsorption of bacterial viruses to polar inorganic substrates should display a number of the same biochemical specificities which characterize their attachment to host bacterial

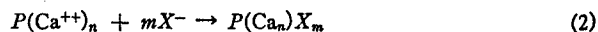
cells; and that virus can be reelected both from host cells to which they have attached themselves, as well as from inorganic surfaces by changing the constitution of the medium so as to reverse the direction of the ionic equilibria involved.

The temperature dependence curve of the virus-cell attachment (Fig. 4) resembles that of an enzyme-controlled reaction rather than the formation of salt-like electrostatic bonds. Further studies which will be described in detail in a forthcoming paper (12) have indeed demonstrated that under the experimental conditions here employed, the curve of Fig. 4 is not a reflection of the initial reversible binding of virus to cell but of a subsequent irreversible metabolic step of the infected aggregate. The rate of the initial reversible step is almost independent of temperature changes (12).

The following set of ionic interactions has been postulated to underlie the process of virus attachment to host cells:



This is the reaction whereby cations are attached to those sites of the "first kind" on the virus surface to give it a configuration matching that of the host cell. The attachment reaction which follows can be represented by:



in which  $mX^-$  represents negative sites distributed over most of the cell surface. Thus, for T1 virus and *E. coli* B, electrostatic and geometrical complementarity is attained in a medium containing  $5 \times 10^{-4}$  M  $\text{CaCl}_2$ . Under these conditions, equilibrium in reaction (1) lies far to the right, so that every phage particle is in the reactive form. The enormous rapidity of the reaction under these conditions is due to the fact that both reactions (1) and (2) partake of the nature of ionic double decomposition, in which a very high collision efficiency is the rule. In this way, the rapidity of activation of this system by  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$ , as well as the high rate of virus adsorption on cells, is explained.

If lower concentrations of  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$  are employed, reaction (1) proceeds less far to the right, so that the concentration of  $P(\text{Ca}^{++})_n$  present at any time is reduced and hence the rate of the attachment reaction is depressed. These considerations explain why the attachment rate in Fig. 2 first rises as the  $\text{Ca}^{++}$  concentration is increased, and then levels off.

This mechanism of the activation reaction (1) involving attachment of cations to sites of the "first kind" probably involves specific sites on the cell surface as well as on the virus. The role of the virus has been emphasized in this presentation because

the known ability of these bacteriophages to undergo reactions with metallic cations in this same concentration range makes it likely that at least some of the sites of the "first kind" are on the virus. Thus, in distilled water T1 undergoes a definite reaction with  $\text{Ca}^{++}$  ion in a concentration of  $10^{-4}$  M to  $10^{-3}$  M, which determines the ability of the subsequently infected cell to produce a plaque when plated in nutrient agar (7). Adams (10) also found that a concentration of  $10^{-3}$  M of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , or other divalent ions, protected T1 from spontaneous decomposition in aqueous solution. Concentrations lower than  $10^{-4}$  M produced no protective action. Of especial significance for the present considerations is the discovery by Adams that monovalent ions like  $\text{Na}^+$  could also prevent this inactivation of the virus, but much higher concentrations of the univalent ions are required. This observation strikingly parallels the fact here described that univalent cations can duplicate the effects produced by the divalent ions on the attachment of T1 to cells, provided that much higher concentrations are employed (Fig. 2). The higher concentration of univalent ions required to produce any of these effects may be taken as an indication of a weaker binding by these ions to the virus groupings.

The fact that T1 virus also requires  $10^{-3}$  M  $\text{Ca}^{++}$  or  $10^{-2}$  M  $\text{Na}^+$  for optimal attachment to glass filters is in accord with this hypothesis. The glass filter experiments also suggest that the inhibiting effect on cell attachment of an excess of  $\text{Ca}^{++}$  (descending branch of Fig. 2; ion-binding at sites of the second kind) is due, at least in part, to blocking of cell attachment sites, since this phenomenon does *not* occur in the glass system. This reaction may be represented by:



It is required that this reaction have a smaller binding energy than that by which  $\text{Ca}^{++}$  unites with the virus, (1), since inhibition begins to be significant only after a concentration of  $\text{CaCl}_2$  greater than  $5 \times 10^{-3}$  M is attained in solution, whereas maximal activation by  $\text{Ca}^{++}$  occurs in one-tenth this concentration. Monovalent ions require still higher concentrations to compete effectively with the activated virus for the  $X$  sites on the cell. Experiments with radioisotope tracers have been carried out which support the view that inhibition of virus invasion is possible by blocking of *cellular* attachment sites by excess cation (12). The fact that the maximal rate of virus attachment to cells attainable in the presence of  $\text{Na}^+$  is significantly lower than that possible with an optimal concentration of  $\text{Ca}^{++}$  or  $\text{Mg}^{++}$  would indicate that the monovalent ion begins to attach to sites of the "second kind" before it has saturated all of those of the "first kind."

The picture which emerges from these considerations portrays the virus as a molecule with specific groupings distributed over its surface which are capable of uniting reversibly with ions from solution. The number of ions of

any kind which is attached at any moment to the virus is a function of the binding energy of each species for the various sites at which linkage is possible, and the concentration of this ion in the medium. Viruses with different host-cell specificities would differ in the geometrical distribution of the ion-binding sites on their surface, and in the binding energies of these sites for specific ions. Thus, when a mixture of different viruses is placed in the same medium, different electrostatic configurations are established on their surfaces, which may determine their ability to become attached to a given host cell. The experiments of the present study have emphasized the roles of certain cations, but there is every reason to expect that the associated anions are also actively participating in this reaction.

The general interpretation here proposed is not affected by the existence of virus strains which require an organic cofactor like *l*-tryptophane in order to attach to a cell. Such molecules may themselves furnish the sites for attachment of the ions which make possible the invasive process. It is noteworthy that tryptophane loses its cofactor function if its strong ion-binding groups—the  $\alpha$  amino group or carboxyl group—are removed (6). Indole even becomes a potent competitive inhibitor for tryptophane in this reaction (13). Since indole contains the same condensed aromatic ring system as the normal cofactor, but lacks the highly polar side chains at which ions could become strongly bound, it would appear that the initial attachment of tryptophane to the virus occurs through the structural elements of the ring system which are common to both tryptophane and indole. In the case of tryptophane, the resulting structure, after having bound the proper numbers of ions at its carboxyl and amino groups, could provide a suitable configuration for attachment to a host cell, whereas indole could not. The theory here proposed would require that even in the presence of an excess of tryptophane, the deficient virus mutant still display characteristic salt requirements before attachment to cells can be initiated. We have found this indeed to be the case.

Ionic effects like some of those described here have been found also to operate in the case of animal viruses. Davenport and Horsfall (14) have recently reported that solutions containing low concentrations of electrolytes inhibit attachment to erythrocytes and to lung particles of both pneumonia virus of mice and of influenza virus. Such solutions also caused redissociation of the former virus from lung particles to which it had been previously adsorbed but retarded elution of the latter. In fact, increase in the elution rate of influenza virus was accomplished by increasing, rather than decreasing the salt concentration. These experiments led the authors to suggest that the attachment of mouse pneumonia virus to cells may involve formation of a complex with the "nature of a weak salt." They postulated a different kind of attachment mechanism for influenza virus, however, at least partly because of its different behavior in the elution experiments.

The detailed theory developed in the present paper can explain equally well this behavior of both influenza and mouse pneumonia viruses. From the nature of the electrostatic interactions postulated, one would predict that refection by high salt concentrations would occur whenever a curve of the general nature of Fig. 2 (*B* curve) characterizes the invasive action, provided it is possible to achieve a salt concentration such that ions from solution can capture the sites of the "second kind." We have found viruses which do not display the descending branch of the curve of Fig. 2, in high salt concentrations. Cell attachment of such viruses should not be reversible in solutions of high ionic strength. Studies of this kind with a variety of viruses are now in progress.

The formulation proposing the existence of two different kinds of sites at which cations may attach, in the virus-host system, would lead to the expectation that specific cations should exist which inhibit the invasion of a host cell by any virus displaying an ionic pattern like that of Fig. 2. Such an inhibitor would be an ion whose binding energy to the sites of the second kind is *greater than* its energy of union to the sites which promote invasion. Such an ion should act as a competitive antagonist to the ions normally promoting attachment. It has been possible to demonstrate such a specific action for the case of Zn, and studies with radioactive tracers have confirmed the predictions concerning its binding energy. These studies will be reported in the second paper of this series (12).

The present observations and the theoretical considerations employed to explain them are suggestive of the mechanism of the metal-catalyzed union of enzyme and substrate studied by Emil Smith, particularly with the dipeptidases (17). Perhaps the most striking difference in the behavior of the two systems is the fact that the activation by metals of the virus-host cell system is an extremely fast reaction, while that of peptidase-peptide systems is slow. This difference may be an expression of the predominance of primary ionic interaction in the virus case, and of coordination forces in the enzymes studied by Smith.

The mechanism which has been proposed here for the primary aspects of virus-cell interaction, and the specificity thereof, differs in emphasis from the physicochemical explanation for biological specificity which has been so successful in explaining the antibody-antigen reaction (15) in that it has been necessary to visualize a mechanism utilizing somewhat stronger forces between the elementary units on the two reacting surfaces. This necessity arises in the virus system because its tremendously high reaction velocity cannot be explained on the basis of very weak binding forces, like Van der Waal's forces, operating between the individual elements on the two bodies. Such very weak forces can provide a model exhibiting a high degree of specificity, since their very great attenuation with distance requires a high degree of geometrical correspondence between the two surfaces, in order for sufficiently extensive atomic contacts

to be established to form a stable union. However, this very requirement of close steric fitting demands that only a small fraction of the random collisions can be effective. In order to relieve this difficulty, Anderson (16) has postulated the existence of "small specific projecting elements" on the host cell or the virus, which might bring about a rapid and specific reaction. There is no experimental evidence for the existence of such structures. In the kind of interaction here postulated which involves linkages of ionic character, virus-host specificity is controlled by the pattern of electrostatic forces achieved by the attachment of small ions to specific sites on the two surfaces. A high collision efficiency results from the fact that (a) strong electrostatic forces would tend to orient the two complementary configurations during their period of approach in the course of a collision, and (b) even if the first collision engaged only a fraction of the maximum number of groups capable of interacting on the two surfaces, the strong binding forces would tend to hold the bodies together long enough for a more favorable adjustment of position to take place.

#### SUMMARY

T1 virus does not attach to its host cell, *E. coli* B, in distilled water. By the proper addition of salts the rate of attachment can be adjusted to any desired value up to the maximum limit set by the diffusion rate of the virus.

Salts of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Ba}^{++}$ , and  $\text{Mn}^{++}$  bring about a reaction rate representing 100 per cent collision efficiency in a concentration of  $5 \times 10^{-4}$  M. Both greater and smaller concentrations depress the attachment velocity.

Salts of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ , and  $\text{Li}^+$  display a similar pattern but require a tenfold greater concentration than that of the previous group to produce the same effect. Moreover, the maximum velocity attainable in solutions containing only monovalent cations is only half that achieved by the divalent salts.

The trivalent cations  $\text{Al}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{3+}$  permanently inactivate the virus.

Activation by  $\text{Mg}^{++}$  of an inert mixture of virus and host cells in distilled water is so rapid as to be beyond the limit of the resolving time of the experimental procedure, which is 20 seconds.

The temperature dependence curve of virus-cell adsorption exhibits a maximum at 37°C. and falls to a value representing approximately 3 per cent collision efficiency at 1°C. Identical curves are obtained in nutrient broth and in  $10^{-3}$  M  $\text{MgCl}_2$  solution.

Bacteriophage can be quantitatively adsorbed on to glass filters. In a study of several viruses this attachment reaction was found to require the same cofactors—both organic, like *l*-tryptophane, as well as inorganic—which each specific virus required for its attachment to its host cell. The suggestion is made that the attachment of viruses to these filters is a useful model for their attachment to host cells.

Virus attachment to glass filters is reversible. Such adsorbed virus can be

recovered almost quantitatively by washing the filter with a solution in which the attachment reaction does not occur.

Virus attachment to host cells is similarly reversible at least in its primary step. Distilled water at 0°C. can produce almost complete liberation of T2 virus from host cells infected in 0.02 M NaCl solution.

Two significant differences between the behavior of glass filters and host cells toward T1 virus are: (a) an excess ion concentration fails to inhibit virus attachment to the glass as it does to the host cell; and (b) no decrease in efficiency of attachment to glass occurs at low temperatures. These facts suggest that the inhibiting action on the infective process of excess cations and low temperatures involves chemical groupings on the cell surface, rather than on the virus.

There is no detectable attachment whatever of T1 virus to *E. coli* cells specifically resistant to it, though still susceptible to other viruses. This experiment indicates that the ion-controlled attachment forces here considered are involved in the host-virus specificity.

This conclusion is strengthened by the fact that several different viruses with different host-cell specificities have different ionic requirements for cell attachment.

All of these observations lend themselves to explanation by a mechanism which pictures an initial addition reaction of cations to specific sites on the surface of the virus in particular, and possibly also of the host cell. Two complementary electrostatic configurations are so produced which can unite in a reaction with a high biological specificity, which yet exhibits 100 per cent collision efficiency. An excess of ions may cover up some of the attachment sites and so inhibit the reaction. By this picture the specificity of virus-cell invasion depends upon the binding energies of sites on both bodies for various ions, and the distribution of these sites over the two surfaces.

Possible relationships of such a process to other biological systems are discussed.

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