JOURNAL OF VIROLOGY, May 1974, p. 1067-1074
Vol. 13, No. 5

Printed in U.S.A.

# Interaction of Mycoplasma with Viruses

## I. Primary Adsorption of Virus Is Ionic in Mechanism

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Received for publication 14 January 1974

We have studied the adsorption of the Gourlay *Acholeplasma* virus MVL-1 to the host cell *Acholeplasma laidlawii* JA-1. Successful adsorption depends primarily on some unknown action of the serum factor in the medium, but evaluation of various physical parameters indicates clearly that given this factor the kinetics is pseudo first order ($K = 3 \times 10^{-9}$ cm$^3$/min) and the mechanism ionic. Chiefly important are the ionic strength of the cation and pH (optimal $Na^+$ = 0.08 M, pH = 6). The system seems indifferent to whether the cation is Na, K, $NH_4$, Ca, or Mg. There is little effect of temperature over the range 0 to 42 C. The diffusion constant of the virus, calculated from its geometry or its maximum adsorption rate, is consistent with its reported size and shape.

As the smallest known autonomous organisms, mycoplasma (formerly called PPLO) are inherently interesting (biology recently reviewed by Maniloff and Morowitz, 15), but particularly so because they are responsible for a number of known diseases in humans and other animals (10) and are suspected as possible etiologic agents in other diseases. To the biophysicist and biochemist the organisms offer the additional property that they are delimited only by a cell membrane, in most ways a typical membrane but with the great advantage that it is uncomplicated by the presence of a cell wall. The lipid composition of the membrane can be varied over wide limits by raising suitable strains in artificial media containing various fatty acids, etc., and mycoplasma have been the subject of a considerable amount of research on membrane structure and function (see Razin, 18).

A virus capable of attacking *Mycoplasma laidlawii* (now usually called *Acholeplasma*) was isolated by Gourlay (4) who has reported many of its properties (1, 5, 8) including the fact that it contains DNA. Maniloff and Liss (14; Bacteriol. Proc., 1971, p. 41, G107) and Gourlay (6, 7, 9) have since found a variety of viruses for *Acholeplasma*, suggesting a large family with somewhat diverse properties. Analogy with the exhaustively studied bacterial viruses suggests that the mycoplasma viruses may provide approaches to important information concerning the biochemistry, molecular biology, and genetics of the parent cell. To us, the virus offers the immediate possibility of serving as a probe of the structure and function of membrane; the present work represents a first step in studying the interaction of the original Gourlay virus, MVL-1 (Mycoplasmatales virus-*laidlawii*-1), and the indicator cell *Acholeplasma laidlawii*.

The structure of the virus MVL-1 is "bacilliform" (a cylinder with rounded ends) having no apparent organ of attachment (1). Thus, the elaborate and elegant penetration mechanism of the tailed phages seems prima facie unlikely with this virus. Although the membranous covering of the host cell might suggest analogy with those animal cells that engulf viruses (cf. Goodheart, 3), without evidence one would hesitate to ascribe such a mechanism to a cell as small and primitive as the mycoplasma. The present study is concerned with primary attachment; the result seems a clear indication that with mycoplasma, as with bacterial and animal cells, the initial attachment proceeds by an ionic mechanism.

These results were presented in a preliminary way at the 1973 meeting of the American Society for Microbiology by D. Fraser, abstract, p. 67.

## MATERIALS AND METHODS

**Acholeplasma.** The strain used for these experiments was *A. laidlawii* JA-1, a naladixic acid-resistant mutant (14) of *A. laidlawii* BN1 (4). This strain was chosen because Maniloff and Liss (personal communication) found it a better host for plaque formation than the original Gourlay strain.

**Virus.** Mycoplasmatales virus 1 (MVL-1) isolated by Gourlay (4) was used for all experiments. Virus

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stocks were prepared by washing confluent plates with phosphate-buffered saline (pH 7.4) for 6 to 8 h at 37 C as suggested by Maniloff and Liss (14). The wash was then filtered through membrane filters (0.22 $\mu$m pore size; Millipore Corp.). The titers of such stocks were usually $10^{10}$ to $2 \times 10^{10}$ PFU/ml.

**Media.** Tryptose broth (TB) was supplemented with 1% glucose (TBG) as suggested by Maniloff (13), but fetal calf serum (FCS) heated at 56 C for 30 min was used at 10% in place of the PPLO serum fraction, for reasons discussed below. It was designated TBGS medium and was used for growing the *Acholeplasma*. Agar plates for virus assay were prepared by adding to standard, cast-glass petri plates, 12 ml of TBGS with 1% agar (Difco, Noble agar). Blood agar plates for titering the *Acholeplasma* were the same but with the addition of 10% sheep blood (13). It seems unimportant whether the blood agar plates have PPLO fraction or FCS.

**Acholeplasma culture.** The host cells seem to grow best in unshaken, capped bottles at 37 C. The number of viable cells per milliliter was determined by colony formation (CFU) on sheep blood plates as suggested by Maniloff (13).

**Virus assay.** *Acholeplasma* lawns were prepared by spreading 0.7 ml of a fresh 17-h culture of JH-1 on agar plates. The plates were then incubated at 37 C for 2 to 3 h with the lids ajar. These young lawns were infected by adding 0.2 ml of virus suspension which was distributed evenly by tipping the plate back and forth. The clear plaques which resulted were counted after incubation at 37 C for 16 to 18 h. All platings were done in duplicate.

**Virus adsorption.** Freshly growing liquid cultures of JA-1 *Acholeplasma* at about $10^8$ to $2 \times 10^8$ CFU/ml were centrifuged at 15,000 rpm for 10 min. The pellets were resuspended in one tenth the original volume of TBGS. This suspension was titered on blood agar plates after appropriate dilution and diluted 1:100 into the adsorption tubes containing the menstruum required for the experiment, giving about $10^8$ CFU/ml. A suspension of MVL-1 was diluted and mixed with JA-1 in prewarmed adsorption tubes at 37 C so that each tube contained per milliliter about $10^8$ cells and $10^6$ viruses except as otherwise noted. All experiments were at 37 C and pH = 7.5 except as otherwise noted. The tubes were vigorously mixed ("Vortex") and incubated statically in a 37 C water bath. Samples of 0.1 ml were removed at specified times and immediately diluted 1:100 in TB. A sample of 1 to 2 ml of this dilution was drawn into a syringe and then filtered by positive pressure through a sterile 0.22-$\mu$m Sartorius filter by using a Millipore Swinnex adapter. We have shown that virus is then recovered quantitatively in the filtrate while the cells are effectively excluded. The filtrate was diluted further, and 0.2 ml of the final dilution was plated on 2- to 3-h lawns of JA-1. A virus control of MVL-1 alone was also filtered and titered, and the unadsorbed virus was expressed as a percentage of this control. The same result, with less efficient exclusion of cells (1% left), is obtained by sampling the supernatant fluid after centrifuging at 10,000 rpm (approximately $12,000 \times g$) for 15 min.

**Optimum ion concentration.** Solutions of NaCl, KCl, and $NH_4Cl$ were prepared to provide cation concentrations ranging from 0.01 to 0.3 M and of $MgCl_2$ and $CaCl_2$ to yield $Mg^{2+}$ and $Ca^{2+}$ ranging in molarity from 0.003 to 0.1 M. All were buffered with the addition of Tris-succinate to 0.005 M. The pH was adjusted to 7.5 by using N HCl. Adsorption in these solutions was measured by removing 0.1-ml samples and diluting and filtering 5 min after adding the *Acholeplasma* and MVL-1 or by plating at various times for kinetic studies.

## RESULTS

**Protein in the medium.** At the outset, these experiments were plagued with a mystery that has not yet been resolved. Using conditions based on the work of Maniloff and Liss (14) and their TBGS medium, we found no measurable virus adsorption. Since both Maniloff and Liss and Gourlay (8), using rather different media, had reported essentially quantitative adsorption in 3 min, many variations of the ingredients of the basic medium and their manufacturer's lots were tried, but to no avail. Discussion with Liss revealed that by luck our experiments and his were virtually identical in detail. The protein component (Difco, PPLO fraction) had seemed most obviously suspect, so we tried two batches but without improvement. Finally, a sample of our medium was sent to Liss who reported (personal communication) that using it he also got no adsorption and that cells grown in our medium required overnight regrowth in his supposedly identical medium to recover. Attempts to acquire from Difco further information and/or other batches of PPLO fraction were essentially fruitless, but in the meantime we found that substituting FCS yielded cells with good adsorptive characteristics. We confirmed and extended Liss' finding that cells in our PPLO medium seemingly grow at the same rate as in the FCS medium, but recover only partial virus-adsorbing ability in 5 h after transfer to fetal calf medium and full ability after regrowth overnight. (Our cells have approximately 2.5-h doubling time in log phase.) Heating the PPLO fraction 1 h at 56 C (standard procedure in the Maniloff-Liss laboratory) gave no improvement. Cells grown in TBGS containing FCS and transferred to an identical medium but substituting Difco PPLO fraction, either heated or unheated, gave good immediate adsorption, but the cells slowly lost this characteristic. FCS works as well heated or unheated, but newborn calf serum, in a single experiment, seems less effective. Cells were grown in 10, 3, and 1% FCS medium; the unadsorbed virus in 5 min was 13, 26, and 88%; hence our decision to standardize on 10% heated FCS.

**Adsorption kinetics in TBGS.** Initial experi-

ments were performed on the kinetics of adsorption of MVL-1 on JA-1 in the TBGS medium in which the "S" represents FCS. As can be seen in Fig. 1, the kinetics does not appear strictly pseudo first order in that the rate slows once the unadsorbed virus drops below 10%. The question of whether the process really is pseudo first order is most simply answered by calculating the "first order constant," K, from initial slopes. Unless cell sites are limiting, its value should be constant regardless of cell concentration, as stated in the well-known pseudo first order adsorption kinetic relationship: 2.3 log $V_o/V_t$ = K [C] $t$. $V_o$ and $V_t$ are the virus concentrations initially and at some time, $t$, min, with adsorbing cells of concentration [C] cells/ml. The results of one experiment are plotted in Fig. 2; the constants, K, calculated from initial slopes from this and another similar experiment are given in Table 1. Clearly, within the error of the experiments, K is a constant; the process is thus basically pseudo first order.

A possible explanation for the falling-off of adsorption rate might be inhomogeneity of the virus with respect to adsorption. Figure 1 describes an attempt to enrich for genetically poorly-adsorbing viruses by selecting twice from those left after prolonged exposure to cells in physiological saline (to avoid or minimize virus reproduction in the cells). Since the resultant virus shows no difference in adsorption kinetics our tentative conclusion is that the drop-off in adsorption is not a genetic property of some viruses in the stock. One might assume that the virus stock is physiologically variable with respect to adsorption, i.e., every stock contains a certain proportion of viruses genetically no different from the others but through some accident of assembly, for example, less capable of adsorption. In the experiment of Fig. 2 the

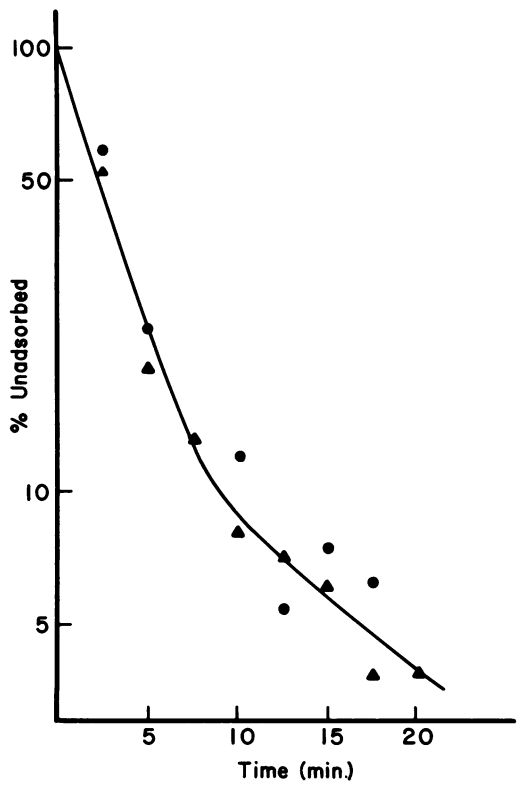


FIG. 1. *Kinetics of virus adsorption in TBGS medium. Symbols: ●, MVL-1; ▲, "slow adsorbing virus" prepared by enriching for unadsorbed MVL-1 through two cycles of adsorption for 1 h in 0.1 M NaCl, pH = 8. Acholeplasma, 1.0 × 10⁸ CFU/ml.*

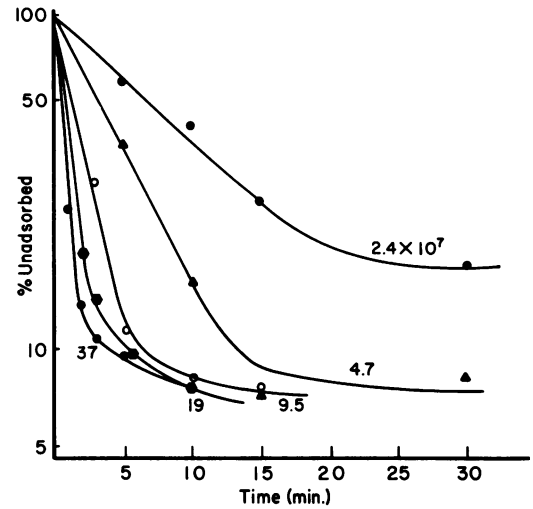


FIG. 2. *Virus adsorption kinetics in TBGS with various concentrations of Acholeplasma. Symbols: ●, cells at 2.4 CFU/ml; ▲, 4.7; O, 9.5; ◆, 19; ●, 37 × 10⁷. MVL-1 initially 6 × 10⁵ PFU/ml.*

TABLE 1. *Virus adsorption to mycoplasma initial slopes of presumed 1st order kinetics*

| Concn of mycoplasma (× $10^{-7}$) | Rate constant (× $10^{9}$) (min) |
|---|---|
| Expt 1 | |
| 5.3 | 2.0 |
| 11.0 | 2.7 |
| 21.0 | 1.9 |
| 42.0 | 2.0 |
| 84.0 | 3.6 |
| Avg. = 2.4 ± 0.7 | |
| Expt 2 | |
| 2.4 | 3.5 |
| 4.7 | 3.8 |
| 9.5 | 4.2 |
| 19.0 | 3.6 |
| 37.0 | 2.7 |
| Avg. = 3.6 ± 0.5 | |

cell concentration for each curve differed, but the amount of virus remained constant. Thus, if a certain proportion of virus is poorly adsorbing, the curves, regardless of initial slope, should change to a slower rate in such a manner that back extrapolation of the end slope should reveal the constant proportion of such defective particles.

Although the results shown might seem to negate this hypothesis, we feel that they are not decisive for several reasons. (i) We have too few points in the slower part of the kinetics. Thus the later slopes are to a considerable degree indeterminate or, at best, depend on a point or two in an experiment showing considerable scatter. (ii) In the experiments with slower kinetics we feel that we are running the risk that progeny viruses may already be appearing, totally vitiating any adsorption kinetics at the later times. We have done further experiments designed to settle this interesting question and they do suggest that there may be a constant proportion of slow adsorbers, but the data become weak just as things get interesting. We have not pressed the point because our intent is to move from cells to isolated membranes where the kinetics should be free of such temporal complications. The possibility that the apparent slowing of adsorption is totally attributable to released progeny virus seems remote in view of the observation that with high cell concentrations (Fig. 2) the "slowdown" occurs in as little as 3 min.

Another possibility is that the cells have a limited number of good adsorption sites and that kinetic slowdown occurs when these are filled. The data of Fig. 2 might seem to indicate that at the higher cell concentrations (higher ratio of cells to virus) adsorption proceeds further before slowdown. This hypothesis was ruled out, however, by an experiment with viruses initially at 2.5/cell compared to viruses at 1 per 200 cells. Any difference in kinetics was within experimental scatter. A suggestion that the sites are to some extent limiting, however, is seen in Fig. 3 in which a UV-killed virus was added at 10 particles per cell. Any attempt to derive from this experiment quantitative data on the number of available cell sites depends on assumptions concerning UV damage to adsorption sites on the virus. Comparison of the slopes, however, shows that the UV-irradiated virus slowed adsorption in both parts of the experiment by a factor of three. On the assumption that the damaged virus adsorbs as efficiently as live, one can calculate the number of good sites per cell to be about 10.

Another possible interpretation—that adsorption is reversible—seems contraindicated by this last experiment, since one might expect the live viruses, supposedly reversibly adsorbed, to be released in the presence of the 1000-fold excess of UV-irradiated virus added at 6 min. As indicated by the ratios of slopes, the subsequent adsorption data seem more consistent with a competition for remaining sites than reversible adsorption.

At any rate, our net conclusion from the above experiments is that the kinetics of adsorption of MVL-1 to JA-1 *Acholeplasma* in TBGS at pH 7.5 is pseudo first order with an adsorption constant of approximately $3 \times 10^{-9}$ but that the kinetics is somewhat complicated by an apparent inhomogeneity of the virus stock possibly with the additional complication of a limited number of good adsorption sites on the cell.

**Effects of cations on kinetics.** With the assurance that adsorption in TBGS is pseudo first order, the same technique was used to examine the adsorption in solutions of various cations with chloride as the anion. The results of Fig. 4 indicate that the adsorption follows the same general kinetics in 0.01 and 0.03 M NaCl. We have adjusted the cell concentration in these and subsequent experiments to keep the kinetics in the first order region; thus the K value can be calculated from the unadsorbed virus at a single time. This proved necessary because we have found no satisfactory method for instantaneous measurement of the concentration of the mycoplasma; thus, to be strictly comparable, data at various concentrations, etc., must be measured essentially simultaneously by using a single batch of cells, and full kinetic runs under these conditions are technically too complicated.

In Fig. 5 are typical data for 5-min adsorption in various concentrations of Na, K, and $NH_4$ chlorides. Although there are some annoying experimental anomalies, it seems clear that within the error all of these monovalent cations behave identically, with a maximum rate constant of about $3 \times 10^{-9}$ $cm^3$/min and an optimum concentration at about 0.08 M. The identical behavior with all three cations was confirmed by examining the kinetics (adsorption at 1, 2, 4, 5, 7, and 10 min, about 10% unadsorbed) at 0.1 M concentration of each. Within the error of this rather unwieldy experiment, the results were the same.

Similar experiments were done comparing the rates in various concentrations of Ca and Mg chlorides, with typical results shown in Fig. 6. The optimum rate is not too different ($K = 4 \times 10^{-9}$ $cm^3$/min), particularly in view of the

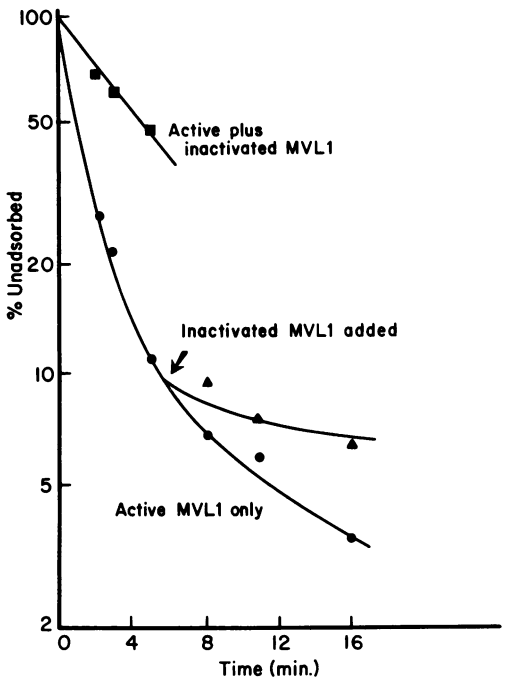


FIG. 3. *Influence of the addition of UV-irradiated virus on the kinetics of adsorption of MVL-1. The "dead" MVL-1 was made by irradiation for 1 h with a UV lamp that reduced survivors to 37% in 100 s; therefore, with virus that had received approximately 36 lethal hits. Live virus, initially $10^6$/ml; UV-irradiated, initially at $1.2 \times 10^9$ particles/ml in adsorption; Acholeplasma, $1.3 \times 10^8$ CFU/ml.*

unusually low apparent *Acholeplasma* concentration. The optimum molarity of the cations, as expected (17), is significantly lower (0.02 M). When kinetics was done with all three monovalent cations and both divalent cations simultaneously at the approximate optimal value for each, the differences were within the errors. Our conclusion, then, is that the adsorption proceeds with essentially equal efficiency in any of these cations at the optimal concentration.

A single experiment was done in which saturating amounts of $Zn^{2+}$, $Cu^{2+}$, and $Fe^{3+}$ were added to 0.1 M NaCl adsorption medium. Surprisingly, none of these often toxic cations significantly changed the adsorption in 5 min.

**Effects of anions on kinetics.** Although anions are seldom of importance in reactions of the type we are considering, comparisons were made of NaCl versus $SO_4$ versus $PO_4$ with the cation at 0.03 M concentration. No significant differences in adsorption in 5 min were seen. A similar experiment with 0.03 M magnesium plus the same anions gave a similar result. Our conclusion is that virus adsorption is indifferent to the anion.

**Effect of pH on kinetics.** In Fig. 7 is shown the effect on adsorption of varying the pH of 0.1 M NaCl buffered, as usual, with 0.005 M Tris-succinate. A rather sharp maximum (approximately at pH 6) is observed, as is consistent with an ionic mechanism.

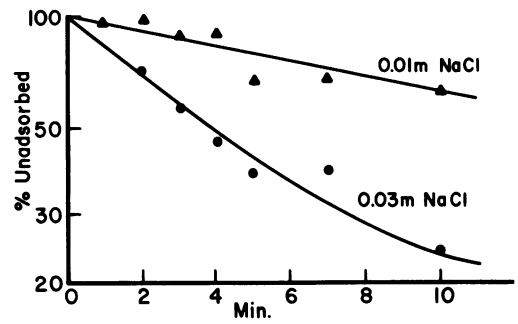


FIG. 4. *Kinetics of MVL-1 adsorption in two concentrations of NaCl. Acholeplasma at $1.0 \times 10^8$ CFU/ml.*

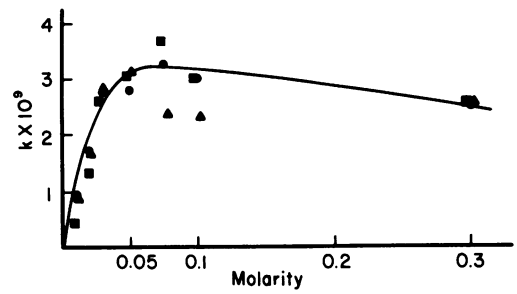


FIG. 5. *Kinetic rate constants of MVL-1 adsorption in various concentrations of monovalent cations. Symbols: ●, NaCl; ■, KCl; ▲, $NH_4Cl$. Acholeplasma, $1.0 \times 10^8$ CFU/ml, pH 7.5.*

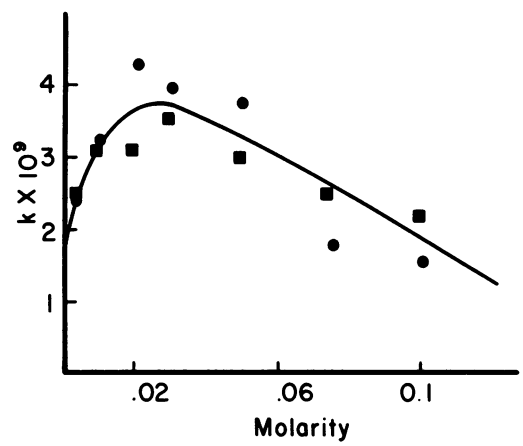


FIG. 6. *Kinetic rate constant of MVL-1 adsorption in various concentrations of divalent cations. Symbols: ●, $CaCl_2$; ■, $MgCl_2$. Acholeplasma, $3.8 \times 10^7$ CFU/ml, pH 7.5.*

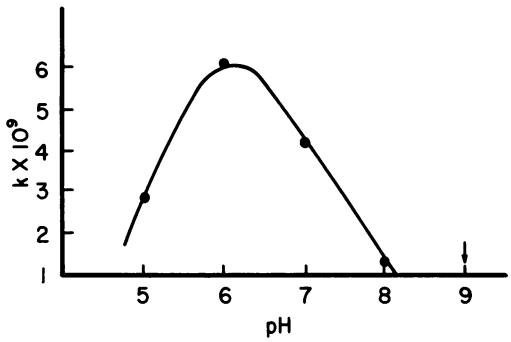


FIG. 7. *Kinetic rate constants of MVL-1 adsorption in 0.1 M NaCl at various pH values. Acholeplasma, 3.5 × 10⁷.*

**Effect of temperature on kinetics.** As shown in Fig. 8, the adsorption rate is little changed with temperature, being only some 50% greater at 42 C than at 0 C. This, also, is consistent with an ionic mechanism and inconsistent with an enzymatic reaction.

## DISCUSSION

All of the data in this work are consistent with the interpretation that adsorption of MVL-1 to the host *Acholeplasma* is by an ionic mechanism and pseudo first order—a result that agrees with those from all other viral systems (see Luria and Darnell [12], p. 193–194, 321). The pH optimum of approximately pH 6 seems to militate against a role of phospholipid in the receptor. The pK of the phosphates in these components (2)—as isolated compounds, at least—is generally low (2 to 3), but this could no doubt be modified greatly in the membrane structure. It is more attractive, a priori, to think of a role of the imidazole moiety of histidine (pK 5.99/25 C).

The apparent finding that only the cation is of importance allows one to compare the divalent with the monovalent to see whether there is evidence of specificity. One might assume that the monovalent cations have only an ionic screening role, whereas the divalent produce a bridging between the receptor and virus. In the studies of Puck et al. (17) on bacteriophage adsorption they found with T1 that the divalent cations were 20× more effective; with T7 some 10× were more effective (12, 22). The implication is thus seemingly in favor of some more specific role of the cation in these instances. Ionic strength, of course, depends on the square of the valence; thus one would expect that the divalent would be four times as effective at a given concentration if there is no specificity. Indeed, within the accuracy of the present experiments, the maximum effect of Ca and Mg seems to occur at 0.02 M, one-fourth the maximum of about 0.08 M seen with the monovalent Na, K, and $NH_4$. Our conclusion, then, is that, in its adsorption to the *Acholeplasma*, MVL-1 virus is interested only in the ionic strength of its cationic environment.

The mystery of the involvement of the serum component of the medium opens so many possibilities that they are not worth discussion in the absence of further information, particularly in view of the fact that the role of the protein required for the growth of most mycoplasma is in itself far from clear (21). It is interesting in this connection to note that in our early experiments we used plates containing the PPLO fraction in forming lawns for observing viral plaques. We found great variability in the plaque counts and morphology which seemed to be overcome by "drying" the plates for several days (at least five) before use. Maniloff and Liss (personal communication) had had similar results. Upon changing to the use of FCS in place of PPLO fraction, we found that this "drying" became unnecessary, and we now suspect that the process involved not a simple drying but some other interaction, possibly denaturation of protein or detoxification of some other component of the medium, which may simply result in better virus adsorption.

The pseudo first order kinetic behavior of the virus in adsorption was, of course, to be expected. And the departure from this pattern as adsorption approaches completion is well-known (12, p. 193) although it usually is observed with phages only after well over 95% have been adsorbed. The causes that we are now suggesting—inhomogeneity of the virus plus a paucity of "good" adsorption sites—we propose to examine more fully with isolated membranes rather than cells to avoid possible complications due to virus growth in the intact cell.

The present results suggest that the maximum value of the adsorption constant, K, is 3

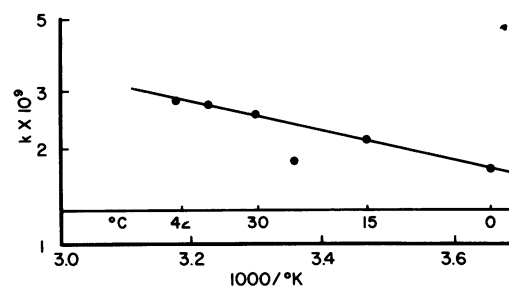


FIG. 8. *Kinetic rate constants of MVL-1 in 0.1 M NaCl at various temperatures. Acholeplasma, 1.1 × 10⁸ CFU/ml, pH 7.5.*

$\times 10^{-9}$ to $6 \times 10^{-9}$ cm³/min. (The average K for a large number of experiments in TBGS is $3 \times 10^{-9}$; the many experiments in cationic solutions yield an average of about $6 \times 10^{-9}$ cm³/min.) This value is, of course, dependent on the accuracy of the measurement of the number of *Acholeplasma* cells. Our experience is that the plating of the cells is not very reliable for reasons discussed by Maniloff (13, p. 132). We also agree with him that measurement of numbers of cells by optical methods is even less reliable. Microscope examination of our cultures shows a mixture of individual cells, small clumps (a considerable proportion), and occasional clumps and/or strings of 10 to 15 cells. Obviously, the clumping of the cells that we have both observed vitiates both optical and colony-counting estimates of cell numbers. Interestingly enough, examination of a large number of kinetic experiments in TBGS and controls for other experiments, also run in TBS, shows that when the cells are grown on a strict schedule, e.g., when the culture is inoculated at the same time each afternoon and removed the same time the next morning, the kinetics is highly reproducible, but the rate constants calculated on the basis of cell plating show considerable scatter. From this we conclude that the estimation of cell numbers is the weakest link in the present work. We have noticed that quite perceptible de-clumping occurs from pipetting and "vortexing" of the cells during dilution. The K of $3 \times 10^{-9}$ to $6 \times 10^{-9}$ cm³/min is strikingly similar to the values reported for T-phages in the classical studies of Puck et al. (17) who found a maximum K value for T2 of $4.5 \times 10^{-9}$ cm³/min. At first sight this might seem puzzling in view of the much greater size of the T-even coliphages, but one must consider that the size of the host cell is also involved. The simplified von Smoluchowski equation used by Delbrück and others since (22) to estimate theoretical collision rate is: $K = 4\pi RD$ where $D$ is diffusion rate of the virus and $R$ is radius of the host cell. Thus, we conclude that the increased diffusion rate of the virus is balanced by the decreased cell radius, resulting in a similar K value. We have considered the host cell as a sphere of 0.9 $\mu$m diameter (15). It is interesting to speculate that clumping of the cells may not distort K significantly. When cells clump, [C] is estimated low, obviously, but this is presumably compensated by an increase in apparent radius, $R$. If we accept our estimate of the adsorption K of $3 \times 10^{-9}$ to $6 \times 10^{-9}$ cm³/min as indicating the maximum value, we can use the von Smoluchowski equation to calculate the diffusion rate of the virus. In Table 2 we compare this rate ($D = 1.2 \times 10^{-7}$ to $1.8 \times 10^{-7}$ cm²/s) with diffusion constants for certain other viruses. The virus closest to MVL-1 in size and shape is probably the bacilliform alfalfa mosaic virus; our rough estimate of the rate for MVL-1 is somewhat greater than that for the slightly larger alfalfa mosaic virus and considerably greater than the rate for the longer tobacco mosaic virus rod of similar diameter.

It is possible, by making certain assumptions, to calculate the diffusion constant from first principles by using only the dimensions of the virus particle. This requires approximating the geometry of rod or bacilliform particles, and a number of different theoretical treatments have been used. The results do not differ markedly in the length-to-diameter region that we are considering, and we used the well-known approximation of Perrin (16) in which the particle is considered as a prolate ellipsoid of major and minor axes $a$ and $b$. We used the electron microscope measurements of Gourlay et al. (5), diameter (d) 15 nm, length 90 nm, hence a/b = 6. The viscosity ($\eta$) of water at 37 C is 0.00695 poise, and we considered 0.007 a sufficiently close approximation to that of the dilute salt solutions of these studies. The general equation for the diffusion constant (19) is as follows: $D =$

TABLE 2. *Viral diffusion constants*

| Virus | Size, shape | Diffusion constant ($cm^2/s \times 10^7$) |
|---|---|---|
| MVL-1 | 15 × 90 nm Bacilliform (5) | 1.2–1.8 |
| Alfalfa mosaic | 18–20 × 60 nm (11) Bacilliform | 1.05–1.1 (11) |
| Tobacco mosaic | 15 × 300 (19) Rod | 0.40–0.53 (19) |
| Turnip yellows mosaic | 22 nm (19) Polyhedral | 1.5 (19) |
| Tomato bushy stunt | 30 nm (19) Polyhedral | 1.15–1.26 (19) |

$kT/f$. For an ellipsoid $a/b = 6$, the geometrical approximation of Perrin, leads to $f = 2.66 \times 6\pi\eta b$ where $b = d/2$. With a Boltzmann constant (k) of $1.38 \times 10^{-6}$ erg/°K and temperature (T) of 310°K, the calculated diffusion constant is $1.6 \times 10^{-7}$ cm²/s—an amazingly (or perhaps fortuitously) good agreement.

## ACKNOWLEDGMENTS

R. N. Gourlay kindly provided the initial stock of the host cell BN-1. We are most grateful to Jack Maniloff and Alan Liss for several discussions that were of great assistance in overcoming technical difficulties and for providing cells and viruses. John Crum extended and confirmed several of the original experiments, and we thank him for his most skillful technical assistance.

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