ELECTROPHORETIC CHANGES IN CELL SURFACES DUE TO ATTACHMENT OF VIRUS PARTICLES

by

LEWIS B. BERNSTEIN

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INTRODUCTION

Microelectrophoresis has been used as a tool for studying bacterial surface properties for over twenty years. With the development of convenient techniques of producing large quantities of uncontaminated viruses, many papers have been published on their effects upon cells (bacterial and blood). With few exception, electrophoretic and microelectrophoretic work with bacterial, viruses, and blood cells, has been directed at determination of various physical and chemical properties of the cells involved. Standardization for the method of investigation of microelectrophoresis as proposed by Moyer (1936) has been generally adopted, and consequent simplification and improvement of its associated optical and electrical systems now places the technique within the catagory of those applicable for routine laboratory use. None-the-less, a general consideration of the technique as one of the methods of definitively characterizing a virus or members of a virus series, such as the T-series of Escherichia coli, has been overlooked. This thesis was planned and executed to fill in this gap.

The investigations within this thesis were limited to consideration of two virus series; Newcastle Disease Virus (NDV) strains Roakin, Manhattan, and California, and the Xp phage series for Xanthomonas pruni. Three phases of study were pursued with each system; (1) differences in electrophoretic mobilities (EPMs) of cells in contact with the various virus strains or phage types, (2) differences in EPM upon addition of

the virus or phage in contact with the cells, and (3) determination of the saturation point of the host cells for its virus or phage. With the Xp-series an additional phase was examined, the separation of a mixed population of phage-infected and non-infected host cells on the basis of EPM.

Some observations will be made, in the case of phages, as to whether attachment to the bacterial host surface occurs as if there were definite receptor points, and if this attachment occurs in a stepwise fashion. It will be attempted to show that the EPM of infected host cells, for a given series of viruses within one system, reflects or is indicative of a relationship between the viruses, and may also be diagnostic of their infectivity (virulence). Thus the possibility of establishing a scale or gradient of infection, for a given series of viruses within one system, based upon the EPM of the infected host cells, is feasible.

A modification of the Swanstrom and Adams (1951) method for the production of high titred stock was developed and will be described. The new method has resulted in production of virus stocks at least 100 times more concentrated than that obtained by the above workers.

REVIEW OF LITERATURE

The literature on surface phenomenon of bacterial cells is widespread and very heterogeneous. Serological, morphological, chemical, physical, and electrical properties of the

bacterial surfaces have been investigated with varying, and oftentimes, conflicting information obtained. Lerche (1953) has given an excellent general review of the phenomenon of electrophoresis, and electrophoresis in relation to microbes. The electrophoresis of proteins, and the chemistry of cell surfaces from a theoretical viewpoint has been thoroughly analyzed by Abramson et al. (1942). At the present time, there has been no published reports as to the effects of virus attachment(s) on the EPM of bacterial cells. Thus, this review of literature, will of necessity, be concerned with the nature of the virus attachment to cells.

Virus particles range in size from 10 mμ to 450 mμ in diameter (Rivers 1952). This size factor places them within the range of colloidal material, and it is not surprising to note that they do behave in many respects as a colloid. Employing the tools of the colloid chemist, such as diffusion, sedimentation, and viscosity constants, the size, shape, and hydration of many viruses have been obtained (Pollard 1953). Electron micrographs have revealed that viruses fall into three major catagories as regards their shape: roughly spherical; rod-like (with an hexagonal cross section); and sperm-like, with nearly spherical heads and quite long tails. The sperm-like (or head and tail) shape seems to be peculiar to bacteriophages solely, the majority of them having this characteristic. Within the T-series of coliphages only T_7 and T_3 have not been shown to have a tail (Evans 1952).

The first step in virus infection of cells is the adsorp-

tion of the virus particle by the susceptible cell. That the adsorption process is completely independent of any later stage in the synthesis of new virus particles was shown by Watson (1950) and Weidel (1950). The former using X-ray inactivated phage found he could get up to 500 inactivated phage particles adsorbed upon each bacterium, and the latter, using cell-free preparations containing "receptor components" of the bacterial membrane, established that the adsorption capacity differed but slightly from the whole intact bacteria. It would thus appear that the adsorption process involves some specific structures on the host and virus. Further strength to this hypothesis has been given by the work of Puck et al. (1951) that no binding of T_1 phage occurs on a specificallymresistant cell mutant.

Schlesinger (1932) first noted that the rate of adsorption of bacteriophage particles specifically and irreversibly to the surface of host cells increased proportionally to the bacterial concentration. Stent and Wollman (1952) have presented evidence that this relationship does not hold true when the numbers of sensitive cells per unit volume is raised above a certain limit. There was a maximum rate of adsorption no matter how dense a bacterial suspension used. They found the rate of adsorption to be dependent upon the temperature. At relatively low concentrations of cells the rate of adsorption by the bacterial cells was found to be proportional to the bacterial concentration. At relatively high concentrations the rate of adsorption became independent of the bacterial concentration. To account for some of

the experimental evidence that they obtained, Stent and Wollman (1952) postulated three alternative theories involving a second step besides collision in the mechanism of bacteriophage adsorption. These three theories are refinements of the simple two-body collision which has been proposed by Delbruck (1940). One theory, termed an "activity-inactivity theory", postulates the phage oscillating between an active and inactive state, and irreversible adsorption occurs dependent upon the state of the phage at the moment of collision with the susceptible cell. A second theory termed an "alternative collision theory" assumes that the bacteriophage particles do not differ from one another in their adsorbability at any instant. However, they may collide with susceptible cells in either a "good" way, which results in adsorption, or in a "bad" way, in which case the phage is held to the bacterium temporarily and reversibly. Following a "bad" collision the phage must be freed from the cell before being elligible for another try at being adsorbed. The third theory, a "surface reaction theory" assumes that the phage and bacteria enter into a reversible attachment after each collision, but that subsequent to this attachment the phage may undergo either an irreversible fixation or free itself.

The difference between the three theories as proposed is merely one of timing as to when a given virus-host cell collision will lead to adsorption of the virus. Under the first theory the ability to adsorb exists prior to collision, under the second theory adsorbability occurs at the instant of collision, and under

the third theory adsorbability occurs some time after collision. The work of Puck et al. (1951) indicates that the "alternative collision" or "surface reaction" theories are probably correct. The earlier experiments of Anderson (1949), who found that no adsorption takes place in phage-bacterial mixtures which are violently stirred by a Waring blender (although this agitation ought to increase the collision frequency and thus the rate of adsorption), can be most easily interpreted from the "surface reaction" theory.

The role of ions in the primary reaction of the mechanism of virus attachment to host cells has been investigated by Garen (1951) and Puck et al. (1951). They found that when the T-series of coliphages were suspended in distilled water, or 10^{-4} m phosphate buffer at pH 6.8, no measurable attachment of of the phages to the susceptible cells occurred. Upon addition of divalent ions such as Ca^{++}, Mg^{++}, Ba^{++}, and Mn^{++}, adsorption occurred with a reaction rate of 100 per cent collision efficiency in a concentration of 5×10^{-4}. Both smaller and greater concentrations depress the attachment velocity. Salts of monovalent ions such as Na^{+}, K^{+}, NH_4^{+}, and Li^{+} showed similar behaviour, but required a tenfold greater concentration than divalent ions, and the maximum velocity of attachment was only one half that achieved by the divalent salts. The trivalent ions of Al^{+3}, Cr^{+3}, and Fe^{+3} permanently inactivated the virus. Activation by Mg^{++} of an inert mixture of virus and host cells in distilled water was so rapid as to be beyond the limit of resolving time of

experimental procedure. This extremely rapid reaction rate between virus and host cells 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. The reaction behaviour observed would not be expected to occur if the reaction mechanisms involved changes in covalency linkages.

It was found that bacteriophage could be quantitatively adsorbed on glass filters. This reaction was found to require the same cofactors, both organic (l-tryptophan for T_4) as well as inorganic, which each specific virus required for its attachment to its host cell. Two significant differences between the behaviour of glass filters and cells toward virus were observed: a) an excess ion concentration failed to inhibit virus attachment to the glass, and b) no decrease in efficiency of attachment to glass occurred at low temperatures. It was suggested, therefore, that the inhibiting process of excess cations and low temperatures involves chemical groupings on the cell surface and not on the virus. The virus attachment to host cells as well as to glass

Medium	Virus Surface	Cell Surface	Result
water			no attachment
10^{-3}m $MgCl_2$			 attachment
10^{-1}m $MgCl_2$			no attachment

Fig. 1. Puck, Garen, and Cline scheme for explaining first phase of virus attachment.

filters was found to be reversible either by dilution or by washing with a solution in which the attachment reaction does not occur.

Puck et al. also found that there was no detectable attachment of virus to cells specifically resistant to it, though still susceptible to other viruses. It would seem then that the ion-controlled attachment forces, which they considered, are involved in host-virus specificity.

Garen and Puck (1951) further extended their concept of viral attachment to cells in this second paper. Evidence was presented which indicated that a second irreversible enzymatic-like step occurred very shortly after the initial electrostatic binding of the virus to the cell surface. Diagrammatically the two steps can be represented as:

(1) $V + C \underset{k_2}{\overset{k_1}{\rightleftharpoons}} VC$ Reversible; independent of temperature; strong electrostatic establishment interactions; forces are specific for a given virus-host combination.

(2) $VC \xrightarrow{k_3} X$ Irreversible; temperature dependent; probably enzymatic establishment of new covalent linkages; forces are specific.

where C= a host cell, V= a bacterial virus, and X= the first metabolic product of the infected unit whose formation is irreversible.

Garen and Puck demonstrated that the second irreversible step showed a definite temperature dependence. By controlling the salt concentration in the case of T_2 they were able to separate the first and second steps. T_2 will attach to E. coli B irreversibly

at 37°C. if the optimum concentration of salt, 0.1 m NaCl, is used. Lower concentrations resulted in a slower attachment rate which was, to a large extent, reversible. These results suggested that ions were required for both the first and second steps of the virus attachment. The authors determined that the second step was the one which resulted in the killing of the cell, and that the initial reversible interaction leaves both virus and host essentially intact. It was found that Zn^{++} specifically blocks the second reaction with T_1. Zn^{++} was found not only to inhibit the second step of invasion, but also prevents Ca^{++} or Mg^{++} from doing so. It appeared to be a competition between the Zn^{++} and the Ca^{++} or Mg^{++} for the same cellular sites. Tracer experiments with the radioactive isotope Zn^{65} showed that an average of 4×10^7 atoms of Zn has to be taken up by each cell to block completely the irreversible step of T_1 invasion. The Zn^{++} was found to be taken up by the host cells rather than the virus. Virus incubated in the presence of zinc still irreversibly attached to susceptible cells, while host cells incubated in the presence of zinc showed only the reversible step.

Ultraviolet radiation was found to inhibit completely the second or enzymatic step. Theoretically this would be expected, since enzymatic activity is to a large extent dependent upon the opening of covalent bonds, and on the intact large protein molecule containing many cyclic aromatic groupings. These types of compounds have a high ultraviolet absorption coefficient. The first step (electrostatic bonding) being dependent upon ionic

interaction, the groups which are responsible for this in biological systems generally have low ultraviolet absorption coefficients.

The resistant action of E. coli B/1 to T_1 was found to be due to the blocking of the second step. Thus, these workers have demonstrated that resistance of mutant cells to specific bacteriophages may be of two types, depending upon whether the first or second steps are blocked.

Treatment of virus and cells with group-specific reagents by Tolmach and Puck (1952) was reported. They found that by varying the H^+ concentration attachment of T_2 to E. coli B was reversibly inhibited at pH 4.8 and pH 10.0 with maximum attachment occurring at pH 6.8. The shape of their pH curve plotted against per cent virus attached to cells suggested that the ionization of carboxyl and amino groups are required for the attachment reaction to occur. Cells treated with carboxyl-blocking groups lost their ability to bind T_2. Cells treated with amino-blocking reagents lost their ability to bind T_1 but not T_2.

Stahmann et al. (1951) demonstrated that synthetic lysine polypeptides can combine with virus (they worked with Tobacco Mosaic Virus) inhibiting its action and causing it to precipitate or aggregate. They proposed the explanation that charged groups on the virus can combine with charged groups on the polypeptide which can then link to a second virus particle causing aggregation. These observations are in agreement with those of Puck and Garen as previously described.

That certain animal viruses had the capacity to attach themselves to the surface of red blood cells and subsequently cause agglutination of these cells was first discovered by Hirst (1942). This reaction has since been named the Hirst phenomenon. The nature of the virus-red blood cell attachment has been extensively investigated, especially by the Australian workers Burnet, Stone, and Ada. Burnet (1952) has written an exhaustive review on the nature of virus-cell interactions in relation to haemagglutination. The principles of bacteriophage-bacterial cell attachment described earlier closely parallel those of virus-red blood cell.

Changes in electrophoretic mobility of red blood cells treated with virus was first demonstrated by Hanig (1948), but extensive and complete studies were carried out by Ada and Stone (1950). They found that a decrease in the electrophoretic mobility could be detected in red blood cells which had been treated with various viruses in order of the viruses receptor gradient scale. It appears from their work that some of the functional groups on the cell surface play only a minor part in determining virus adsorption although contributing equally to the net negative charge. They proposed that there are two types of substrate, A and B, which when destroyed or modified reduce the average EPM. Most viruses act only on the A series, but Newcastle Disease Virus (NDV), Swine Influenza (SW), and Receptor Destroying Enzyme (RDE) act on the B series.

Magill (1951) presented evidence that the forces of attraction

between influenza virus and chicken erythrocytes are governed by an orderly mechanism which effects a proportional distribution of virus between erythrocytes and suspending fluid. The adsorption over a wide range of concentrations of virus indicated compliance with the laws of mass action.

Burnet (1952) has shown that adsorption of virus on red blood cells does not take place unless cations of Ca, Mg, Na, or K are present. A lower concentration of Ca and Mg was required than of Na or K, as with bacteriophage.

METHODS AND MATERIALS

Virus-Bacterial Cell Electrophoretic Determination Procedures

The microelectrophoresis apparatus used in obtaining the experimental data in all experiments was the Abramson modification of the Northrop-Kunitz electrophoresis cell. Electrophoretic mobility measurements were made following the method outlined by Moyer (1936). A Heathkit audio impedence bridge and platinum electrode was standardized with 0.1 N KCl, and was used for measuring the specific conductance of the solutions used. All experimental work was performed at room temperature (24°-27°C.).

The original phage stock and X. pruni culture used in the phage-host cell experiments were obtained from A. Eisenstark, Kansas State College, Department of Bacteriology. The phages used were Xp-1, Xp-2, Xp-3, Xp-4, Xp-5, and Xp-8. The method of preparation of phage stock will be described in the appendix.

The cells used for each determination were prepared by inoculating three 300 ml. flasks containing nutrient broth (Difco), to which an additional 0.5 percent glucose had been added, with a small loopful X. pruni from a nutrient agar slant. The flasks were then incubated at 28° C. for 36 hours. The cells were centrifuged at 2500 rpm for 10 minutes and washed three times in 0.01 m sodium acetate buffer (pH 7.05). X. pruni is a heavily encapsulated organism which tends to clump, especially upon centrifuging. In order to obtain a homogeneous suspension of cells, sterile glass beads were placed in the flask containing the final washed suspension of cells, and then vigorously aggitated for five to ten minutes. This procedure produced a homogeneous suspension and also succeeded in breaking up the diplobacillary and short chain forms of the organisms in the suspension into single cells, which also made possible a more accurate cell count. A 5.0 ml. sample of the suspension was then taken, from which, by the standard Petroff-Hausser bacterial counting procedure, the numbers of organisms per ml. of solution was determined. The remaining suspension was plated on nutrient agar plates and spot inoculated with phage as a check on susceptibility of the host to phage action. The suspension was then stored at 8° C. for four hours before using in the electrophoresis cell. The average bacterial cell count per suspension averaged about 10^9 cells/ml.

The cell and cell-virus suspensions that were ultimately introduced into the electrophoretic cell were prepared in the

following manner for the entire series of Xp phage. The final ratios of phage to bacteria desired were 0:1 (control), 1:1, 5:1, 50:1, 100:1, 200:1, and 500:1. The phage stocks having been previously titred by the standard plaque count method, and the numbers of cells in the suspension counted, it was possible, through appropriate dilutions, to obtain the above ratios of phage to bacteria. The time necessary to run the series of six phages through the electrophoresis cell was well over two hours. Since the burst time of the Xp phages was just under two hours, mixing of the cells and phage could not be done until just before introduction into the cell. It was found that relatively small amounts of phage stock solution (1 - 5 ml.) when added to the host suspension had a very marked depressing effect upon the total resistance (and therefore the conductivity) of the suspension. Wherever possible, the desired ratios of phage to bacteria was achieved with a minimum of phage suspension (never more than 1.0 ml.).

The mixtures of virus and host cells were allowed to react for ten minutes (to obtain maximum adsorption), and then the solutions' specific conductivity measured and recorded just prior to introduction into the electrophoresis cell. The conductivity was also measured at the end of each sample reading. The electrophoretic cell was thoroughly washed with 0.01 m sodium acetate buffer before and after each sample. The mobility of the cells were observed under 210 x magnification over a distance of 32 microns, each cell being allowed to travel 32 microns in one

direction, after which the charge was reversed, and the cells were allowed to travel 32 microns in the opposite direction. The total time (as determined by a stop watch) was divided by two to obtain the observed average mobility for 32 microns. A General Electric Milliamperemeter was attached in series to a 90 volt DC current supply with a variable resistance to measure the current passing through the cell. One hundred time determinations were made on each sample.

Calculation of the actual mobility in terms of microns/sec/volt/cm. was obtained by using the following formula:

$$v = \frac{d\ A}{t\ I\ R}$$

where v= the mobility in terms of μ/sec/v/cm., d= distance in microns the particle traveled in time t (secs.), A= cross-sectional area of the cell in cm^2, I= amperes, and R= resistance in ohms. For example, if the observed average mobility of a single cell were 3.20 seconds for 32 microns, the measured resistance of the suspension 150 ohms, the area of the cell 0.112 cm^2, and the current used 0.8 milliamp., the mobility of the cell observed in terms of μ/sec/v/cm. would be:

$$v = \frac{(32)\ (.112)}{(3.2)(.0008)(150)} = 9.08\ \mu\text{/sec/v/cm.}$$

Virus-Red Blood Cell Electrophoretic Mobility Procedures

The same procedure as outlined under methods and materials for virus-bacterial cell microelectrophoretic determinations were used in the operation of the electrophoretic cell. The

three strains of Newcastle Disease Virus (NDV), Roakin, Manhattan, and California, used were obtained from R.K. Bower, Kansas State College, Department of Bacteriology. The viruses had been grown in chick embryos, and the harvested infected allantoic fluid stored at -10° C. . HA titres for each of the viruses was determined using the standard procedure (Rivers 1952).

The chicken erythrocytes employed were prepared by withdrawing the blood from the bird into a physiological saline solution containing two per cent sodium citrate, in the ratio of nine parts of blood to one part of the citrate solution. The cells were then washed and centrifuged three times in 45 volumes of saline, sedimenting the cells the first two times at 1600 rpm for 8 minutes, and the last time at 1000 rpm for ten minutes. The cells were finally resuspended in 0.5 per cent concentration of the following medium:

½ volume ..... 0.77% NaCl

½ volume ..... 0.908% KH_2PO_4 - 1 part

0.94% Na_2HPO_4 - 2 parts

To characterize strain differences, four solutions were prepared: a control solution containing equal amounts of normal chick allantoic fluid and the above buffered chick red blood cells suspension, and three solutions (one for each of the virus strains) containing equal parts of red blood cells, normal allantoic fluid, and virus suspended in buffer to bring it to an Ha titre of 1280. The solutions were not mixed until just prior to their being introduced into the electrophoresis cell. The specific

conductivity of each solution was measured before introduction into the cell. A constant current was maintained by adjustment of the variable resistance. Measurements of mobility were made at the top stationary level of the cell. The length of time taken by the red blood cells to move 100 microns towards one electrode, after which the current was reversed and the time the cells required to travel 100 microns in the opposite direction was recorded. The total time divided by two gave the average observed mobility of the cells per 100 microns. Each solution was divided into five aliquot portions, and five readings were taken within each portion in each direction. Thus fifty readings for each solution were obtained.

The effect of diluting the virus suspensions (that is, changing the ratio of red blood cells to virus) upon the mobility of the red blood cells, was determined using a similar technique. A control of normal allantoic fluid and red blood cells was run with each determination. Each virus was first adjusted to an HA titre of 1280 by dilution in buffer. Three solutions of each virus and red blood cells were prepared. The differences in the solutions being that the virus was successively diluted in buffer 1:1, 1:5, and 1:50. Each sample consisted of 5.0 ml. of red blood cell suspension, 5.0 ml. of normal allantoic fluid, and 5.0 ml. of the appropriate virus diluted in buffer. The conductivity of each solution was measured before its introduction into the cell, and a constant current was maintained. A 2 ml. sample of each of the virus solutions after they had been run through the electrophoresis cell was collected and stored at -10° C. and

their HA titres were determined at a later date.

EXPERIMENTAL

Differences in Electrophoretic Mobility (EPM) of X. pruni Upon Addition of Varying Concentrations of Xp Phage

The effect on EPM of addition of varying amounts of Xp bacteriophage to susceptible host (X. pruni H-S), thus changing the ratio of phage per bacterial cell, was measured. The results are summarized in Table 1. . A graph representing a plot of the differences in EPM of the various phage-cell ratios minus the control EPMs, versus the number of phage per bacterium is shown in Fig. 2. . Theoretically it was to be expected that upon the addition of phage to a susceptible host there would be a diminution in the host's EPM if the primary nature of virus-host attachment is electrostatic in nature, as the evidence of Garen and Puck (1951) indicated. It was also to be expected that a different measurable EPM would be found as more and more phage attach to these electrostatic sites, thus gradually decreasing the total charge on the bacterial surface which, of course, governs EPM. Both these hypotheses were found to be true as examination of Table 1. will reveal.

Previous workers (Stent 1952, Delbruck 1940, Puck 1951) have indicated that under a given set of conditions there is a maximum total per cent of phage adsorbed from solution by susceptible host cells. By calculations from per cent of total phage adsorbed, they have been able to estimate the probable total number of phage particles that a given virus host-cell combin-

ation can adsorb.

Table 1. The effect on EPM in terms of μ/sec/v/cm. of different ratios of phage/bacterium.

phage per bacterium	Xp-1	Xp-2	Xp-3	Xp-4	Xp-5	Xp-8
control	15.40	17.85	12.29	8.54	10.95	9.58
1:1	15.11	18.01	12.38	8.60	11.04	9.03
5:1	10.50	17.06	12.24	8.46	10.91	7.82
50:1	6.95	17.67	12.14	7.21	10.86	4.59
100:1	6.90	11.00	7.69	6.99	8.95	4.47
200:1	6.25	10.91	7.80	6.86	8.65	4.80
500:1	6.25	10.25	7.57	6.90	8.75	4.34

If there is a saturation point, beyond which a bacterial cell cannot adsorb further phage, it should become evident from no further,change in EPM being detected after the saturation point has been reached, no matter how much additional phage is added. The results of this experiment indicate this clearly. Beyond a ratio of 200 phage per bacterium the EPMs remain practically constant; no statistical difference can be detected except for Xp-1 which reaches its stabile level at 200:1. The saturation point for the other five strains of Xp phage appears to lay somewhere between 100 and 200 phage per bacterium.

The author was unable to achieve a stabile electrophoretic mobility value for X. pruni H-S controls which would agree from day to day, but EPM values within a given series on a given day

did not excede a five per cent coefficient of variation, except with Xp-1, the samples of which reached a coefficient of variation of eight per cent. The instability of the host from day to day was not entirely unexpected though, since it has been observed in other experiments that X. pruni is very unstabile in its susceptibility to phage action as the author had some difficulty in obtaining consistant host range mutants. There appears to be a growth phase relationship, but this has not been thoroughly investigated as yet.

The negative and eratic mobility changes at low ratios of virus to bacterium (1:1, 5:1) especially as exhibited by Xp-2, Xp-3, Xp-4, and Xp-5, can be explained on the basis of probable phage-cell collisions. At these low ratios, the liklihood that a given phage particle will collide with a given cell, and that that cell in turn will not collide with another phage particle is very low. This would leave essentially two populations which chance sampling in the electrophoretic cell may or may not detect. The increased mobility observed in the several cases at these low phage-cell ratios can be thought of, to extent the previous hypothesis, as a result of the tying up of cations in the union of those cells which have adsorbed phage. As a consequence the medium in establishing equilibrium gains some positively charged ions from the surface of the suspended cells. This loss from the cell surfaces of positive ions would tend to make them more electronegative therefore increasing their EPM. It was found also, at these low ratios of phage to cells, that a large number of cells in the population would exhibit a strong polarized effect. They could be

seen to "flip over" when the current was reversed. On the basis of the above hypothesis this can easily be attributed to a loss of cations preferentially from one end of the cell, or to the attachment of the phage in these particular cells having occurred at the cell extremity.

The grouping of the six mobility curves in Fig. 2. into three groups - Xp-1 and Xp-2; Xp-3 and Xp-8; Xp-4 and Xp-5 - closely follows the serological groupings of these six phages as determined by Kirchner (1954). Since the antigenic mosaic of microorganisms has been associated with the genetic constitution of the organism, it is not hard to envision the electrophoretic surface as being genetically controlled. Data on resistant hosts and Xp phage, which will be presented later, will give evidence to sustain this hypothesis.

The total per cent decrease in EPM of host that each phage strain produced upon saturation of the host cells is presented in Table 2. . Stone and Ada (1950) obtained comparable results

Table 2. Maximum per cent decrease in EPM caused by saturation of host cells with the various strains of Xp phage.

Phage strain :	Maximum % decrease in EPM
Xp-1	59.41
Xp-2	42.57
Xp-3	38.40
Xp-4	19.20
Xp-5	20.09
Xp-8	54.69

using various strains of influenza virus and mumps virus. They

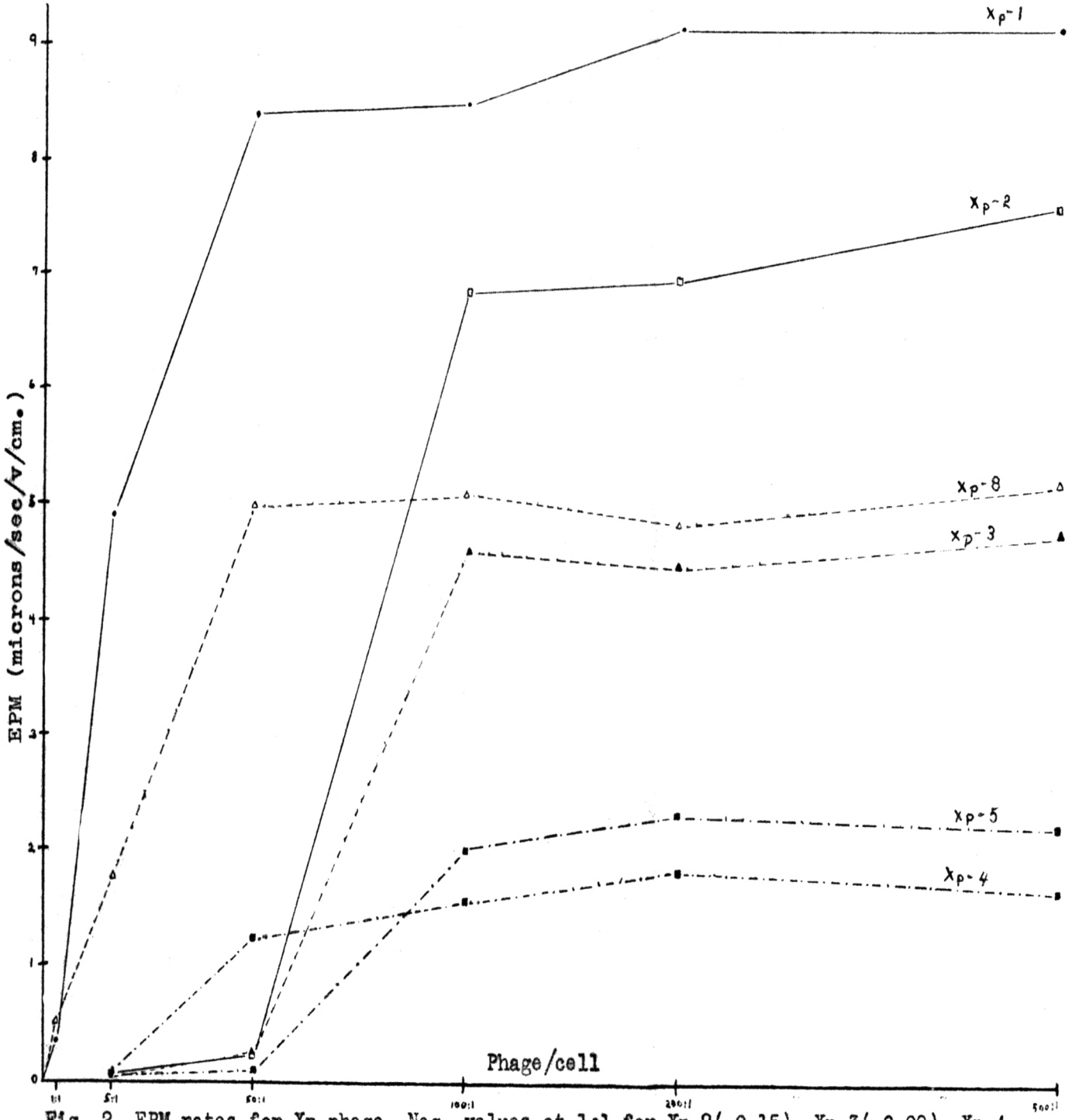

Fig. 2. EPM rates for Xp phage. Neg. values at 1:1 for Xp-2(-0.15), Xp-3(-0.09), Xp-4 (-0.06), and Xp-5(-0.09) are not shown.

were able to calculate and arrange in a linear order decreasing values of EPMs of red blood cells treated with each of the above viruses. The values they obtained were closely related to the receptor gradient order of the viruses they employed.

Differences in EPM of Three Strains of X. pruni

The procedure used was essentially the same as that listed under virus-bacterial cell procedures, the only difference being that no virus was employed. The three strains of X. pruni examined were : X. pruni H-S (a strain susceptible to all the Xp-series of phage), X. pruni H-R (a strain resistant to all the Xp-series), and X. pruni H-L15 (a strain thought to be lysogenic, and susceptible only to the action of Xp-3).

Table 3. Differences in EPM of three strains of X. pruni.

Strain of X. pruni	Average EPM (μ/sec/v/cm/)
X. pruni H-S	9.58
" " H-R	15.69
" " H-L15	11.38

Table 3. is a summary of the results obtained.

Stent and Wollman (1952) and Puck et al. (1951) demonstrated that irreversible binding of virus to a host cell specifically resistant to it did not occur. This evidence leads to the conclusion that in spite of a proper ionic atmosphere, attachment will not occur unless the electrostatic sites have a proper

electrostatic configuration. The nature of the surface of the cell is, in all propability, genetically controlled, thus different strains of an organism could be expected to have different surface configurations. Lerche (1953) demonstrated quite conclusively that different strains of Micrococcus pyogenes var. aureus did possess different EPMs. It was not surprising to find, as Table 3. indicates, a difference in EPM between the three strains of X. pruni examined. The strains used differed only in their sensitivity to phage action. The question then arises, does it follow that there is a relationship between EPM and phage susceptibility, or are variations in surface common in different strains? The answer to both parts of the question is a qualified yes, as witness the intermediate EPM value found for H-L15, a strain which not only carries a provirus (lysogenic), but has been found to be susceptible to the action of Xp-3. It may be that the provirus contributes some factor which influences the electrostatic nature of the cell surface.

The EPM of H-R was not found to differ significantly ($p < .25$) when phage was added to the suspensions even in large quantities. Table 4. shows this quite clearly.

Table 4. The EPM of X. pruni H-R upon the addition of Xp-8.

Ratio virus/bacterium	:	Average EPM (μ/sec/v/cm.)
control		15.69
50:1		15.80
100:1		15.72
10^3:1		15.92

Separation of Infected and Non-Infected Cells on the Basis of Their EPM

The experimental results obtained in the preceeding two sections led to the hypothesis that a population containing a mixture of phage-infected and normal cells could be detected quantitatively by EPMs. The following experiment was performed to demonstrate this. The procedure for preparing and counting host cells as previously described was followed. Two 10.0 ml. samples of the washed and resuspended host cells were then adjusted by dilution techniques (using 0.01 m sodium acetate buffer as diluent) to a concentration of 10^3 cells/ml. . To 15.0 ml. of one of the adjusted cell suspensions (the other adjusted sample having been placed at 8° C. to inhibit further cell division) one drop (approximately 0.1 ml.) of Xp-8 phage titering 5 x 10^{10} phage particles / ml. was added and allowed to stand for ten minutes. The cells were then centrifuged down, washed twice, and resuspended in the same amount of buffer. This process effectively left the cells saturated with phage, removed the excess phage from the suspending medium, and left the cells at the desired concentration of 10^3/ml. . This 15 ml. sample of phage saturated cells was then mixed with 15 ml. of the normal cells which had been refrigerated. The mixture, which now contained a 1:1 ratio of phage-saturated to normal cells, was then introduced into the electrophoresis cell, and readings of 100 cells each were taken. By using this low concentration (10^3 cells/ml.) of cells, only two or three bacterial cells were visible in the microscopic field of the electrophoresis

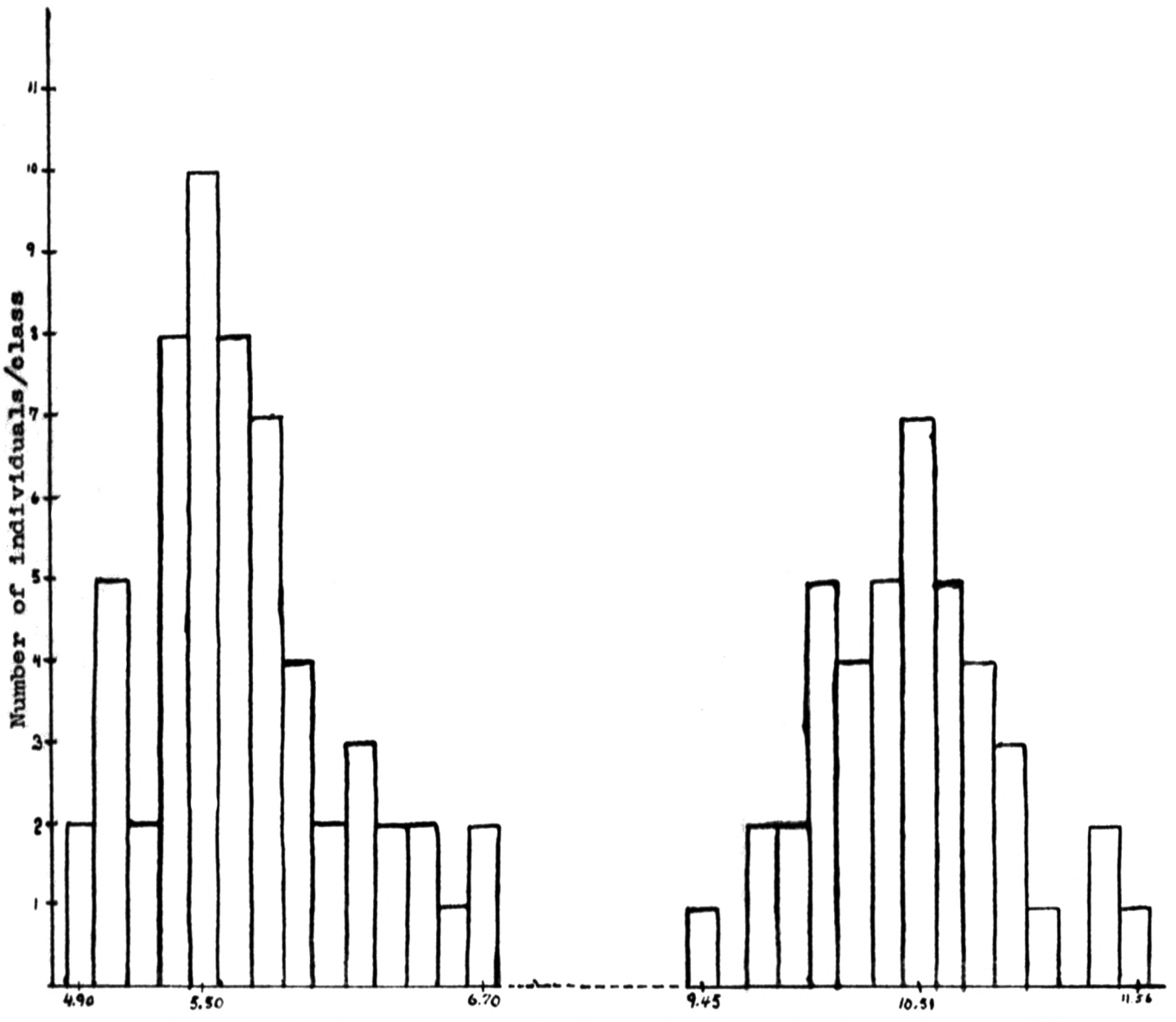

Fig. 3. Histogram showing separation of infected from normal cells on basis of EPM.

cell at any one time, thus enabling unbiased observation to be made, as every cell could be counted.

The results of this experiment are summarized graphically in Fig. 3. . It can be seen that two populations with different means were observed. The faster moving, or non-infected cell fraction composing 57 per cent of the total observed population. Statistical analysis showed the difference in mobilities to be highly significant ($t = 182.55$, $p \ll .001$).

Differences in EPMs of Chicken Erythrocytes Upon Addition of Varying Concentrations of NDV Strains

Earlier workers (Ada and Stone 1950) have reported a decrease in EPM of red blood cells upon addition of various viruses in the order of their receptor gradient series. This experiment was designed to test if varying concentrations of virus added to red blood cells resulted in a decrease in the EPM of the cells. Three strains of NDV were examined: Roakin, Manhattan, and California. The results obtained are summarized in Table 5.

Table 5. EPM of chicken red blood cells upon addition of varying dilutions of NDV strains.

Virus dilution	NDV strains		
	Manhattan	California	Roakin
control	16.68	17.29	14.18
1:50	12.95	11.87 *	13.85
1:5	12.05	11.60 *	11.18
1:1	10.66	11.57 *	10.80

* LSD non-significant

The basis for virus dilution was HA units, the initial virus suspension of each strain having been adjusted to 1280 titre. The

subsequent virus dilutions were made from these stocks. Since haemmaglutination titres are not necessarily proportional to infectivity (and thus to the numbers of "complete" virus units present), no conclusions as to the maximum numbers of particles attaching to a cell can be drawn (as with the phage-bacterial cell EPM studies). The data obtained indicates clearly that the addition of varying numbers of virus particles, whether in the complete or incomplete stage, effectively reduces the EPM of the red blood cells (or total surface charge). That there is a maximum number of adsorbed particles by red blood cells in he case of NDV and influenza virus strain Pr8 has been shown by Bang (1952) for the former, and Ada and Stone (1950) for the latter.

Figure 4. represents graphically the decrease in rate(s) of EPM of the red blood cells with various dilutions of the virus strains. The non-significant change in mobilities found for the successive dilutions of the California strain may be associated with its virulence, since this strain is the only one of the three used in this experiment which will cause death of the birds. The fact that statistically significant successive decreases in mobilities did not occur, even upon dilution of the virus, may be related to the adsorption coefficient for this strain.

Differences in EPM of Red Blood Cells Upon Addition of Various Strains of NDV

The procedure as described previously for the preparation

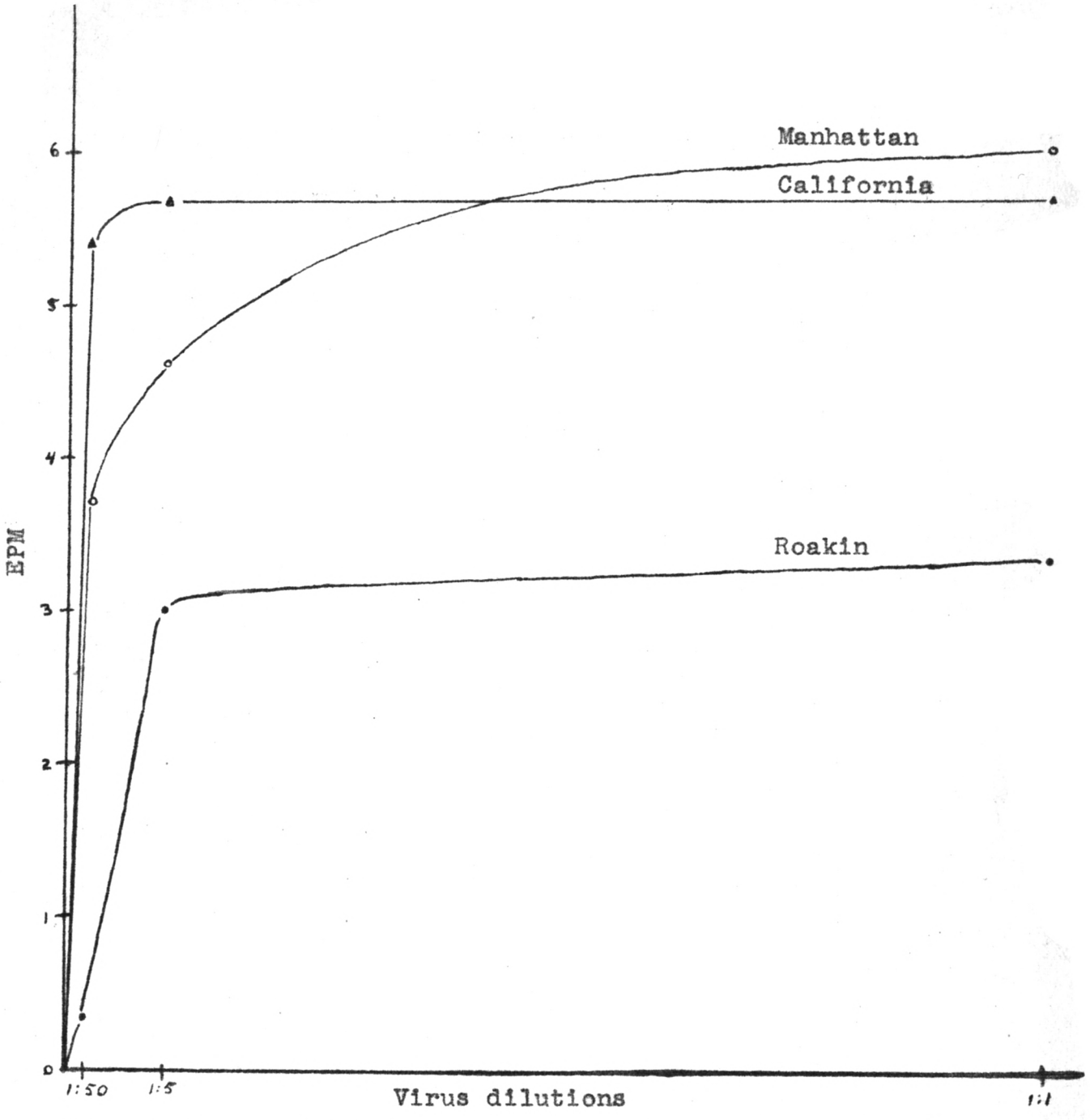

Fig. 4. Rates of EPM of red blood cells treated with NDV strains.

and use of virus-red blood cells in the electrophoresis cell was followed with one modification. The virus stock in this experiment had a HA titre of 2560.

As can be seen from Table 6., the decrease in EPM caused by the Roakin and Manhattan strains did not significantly differ from each other, while the California strain showed a marked decrease from control as well as from the other two strains.

Table 6. Differences in EPM of red blood cells due to various strains of NDV.

Strain	EPM	Max % decrease in EPM
control	10.45	-----
Roakin	6.25	44.01
Manhattan	6.87 *	34.26
California	4.25	59.33

* LSD non-significant

It was noted in all work with red blood cells and NDV that it was necessary to mix the red blood cells and virus solutions just prior to taking mobility readings. If the cells and virus were allowed to remain mixed for longer than fifteen minutes before readings were taken, it was impossible to detect differences in mobilities within strains upon dilutions of the virus. This phenomenon was not entirely unexpected, since the "creeping action" of the haemmaglutinating viruses upon red blood cells has been known for many years. That is, quantities of haemmaglutinating viruses which are known to have very low

HA titres, when tube tested for HA activity, will show agglutination in all tubes if not read before 30 to 45 minutes.

CONCLUSIONS

The results of these experiments further substantiate the current concept that the primary nature of virus-host attachment is electrostatic. The ability to detect a differential EPM upon addition of varying concentrations of virus was found to hold true for both phage-bacterial cell and animal virus-red blood cell systems. That the ability of viruses to attach to cell surfaces is, for the most part, governed by surface charges was clearly demonstrated. But, if surface charges were the only factors operating in attachment of virus and host cell, everything else being equal, the EPM of the host cell would logically be expected to approach zero with the addition of sufficient virus to completely saturate the cell charge. It was found, however, that only a partial reduction in total EPM occurred no matter how many virus or phage particles were added, and that the residual mobility was well above zero. That the stabile level of mobility occurred at a ratio (in the case of X. pruni) of about 200 phage particles per bacterium, occludes the possibility of the mass of phage attached on the surface being large enough to block other phage particles from being adsorbed. Two-hundred Xp phage particles would barely cover one twohundreth (if that much) of the total bacterial surface. This leads to the inevitable conclusion that the electrostatic sites where attachment takes place must have a specific configuration.

The fact that host-range mutants specifically resistant to phage action showed no change in EPM supplements this conclusion, and would also indicate that the fundamental control of phage attachment or adsorption rests in a genetic mechanism.

The two series of viruses (Xp phages and NDV strains) tested each displayed different percentage decreases in EPM when reacted with their respective hosts. The total per cent decrease caused by each strain was found to be consistant. Three strains of X. pruni, which differed only in their capacities to adsorb phage, also showed characteristic differences in EPM. These observations again point to specific configurations within the electrostatic sites and thus to a basic genetic control. One additional bit of evidence for a relationship between surface charge configuration and genotypic constitution of the "organisms" revealed by these experiments, lies in the parallelism between the Xp-phage serological relationships and the total decrease in EPM that they are able to cause. Practical application could conceivably be made of this as a rapid preliminary screening procedure for determining serological placement for new members of an established phage series.

The ability to distinguish virus-infected from non-infected cells in a mixed population of the two was clearly demonstrated. Qualitative and/or quantitative utilization could be made of this as a means of checking suspect infected or lysogenic cultures.

SUMMARY OF EXPERIMENTAL RESULTS

Differential decreasing rates of mobility were observed to occur when varying ratios of X. pruni phage strains to susceptible X. pruni host cells were mixed, and when different concentrations of NDV strains were added to red blood cells.

The total per cent decrease in EPM caused by each strain was found to be characteristic of the strain in both the X. pruni phage system and the NDV system.

The total decrease in EPM of host cells caused by the addition of Xp phage closely paralleled the serological groupings of the phage.

Three strains of X. pruni differing in their susceptibility to phage action were found to have different EPMs.

It was found possible to quantitatively detect the numbers of phage-infected from non-infected cells in a mixed population on the basis of their different EPMs.

An estimate of the maximum numbers of phage particles which can be adsorbed to susceptible X. pruni host cells was found to be 200 phage particles per bacterium.

No change in EPM of X. pruni cells resistant to phage action was found to occur upon addition of Xp phage. Red blood cells which had previously been treated with NDV, and the virus then eluted and removed, also showed no change in EPM upon the addition of fresh virus of the same strain.

The most infective of the three strains of NDV tested

showed a significantly larger percentage decrease in the EPM of chicken erythrocytes than did the other two strains.

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LITERATURE CITED

Abramson, H.A., L.S. Moyer, and M.H. Gorin.
Electrophoresis of proteins and the chemistry of cell surfaces. New York. Reinhold. 1942

Ada, G.L. and J.D. Stone
Electrophoretic studies of virus-red blood cell interaction. I. Brit. J. Exptl. Med. 31:263-274. 1950.

Ada, G.L. and J.D. Stone
Electrophoretic studies of virus-red blood cell interaction. II. Brit. J. Exptl. Med. 31:275-284. 1950.

Anderson, T.F.
Morphological and chemical relations in viruses and bacteriophages. Cold Spring Harbor Symp. Quant. Biol. XI:1-14. 1946.

Anderson, T.F.
The reactions of bacterial viruses with their host cells. Bot. Rev. 15:465-505. 1949.

Bang, F.B. and M.A. Foard.
The effect of Newcastle Disease Virus on chicken red blood cells. I. Amer. J. Hyg. 55:363-372. 1952.

Bang, F.B. and R. Libert.
The effect of Newcastle Disease Virus on chicken red blood cells. II. Amer. J. Hyg. 55:373-385. 1952.

Burnet, F.M. and M. Edney.
Recombinant viruses obtained from double infections with influenza A viruses, MEL and neuro-WS. Aust. J. Exptl. Biol. Med. Sci. 29:353-362. 1951.

Burnet, F.M.
Haemagglutination in relation to host cell-virus interaction. Ann. Rev. Microbiol. 6:229-246. 1952.

Delbruck, M.
Adsorption of bacteriophages under various physiological conditions of the host. J. Gen. Physiol. 23:631-642. 1940.

Evans, E.A.
Biochemical studies of bacterial viruses. Chicago.U. Chic-Press. 1952.

Garen, A. and T.T. Puck.
The first two steps of the invasion of host cells by bacterial viruses. II. J. Exptl. Med. 94:177-189. 1951.

Hanig, M.
Electrokinetic change in human erythrocytes during adsorption and elution of PR8 influenza virus. Proc. Soc. Biol. Med. 68:385-392. 1948.

Hirst, G.K.
Adsorption of influenza hemagglutinins and virus by red blood cells. J. Exptl. Med. 76:195-209. 1942.

Kirchner, C.
The antigenic relationships of bacteriophages active against Xanthomonas pruni. Master's thesis. Dept. Bact. Kansas State College. Manhattan, Kans. 1954.

Lerche, C.
Electrophoresis of Micrococcus Pyogenes Aureus. Acta. Path. et Microbiol. Scandinav. Suppl. 98. 1953.

Magill, T.P.
The sorption of influenza virus by chicken erythrocytes. J. Exptl. Med. 94:31-43. 1951.

Moyer, L.S.
A suggested standard method for the investigation of electrophoresis. J. Bact. 31:531-545. 1936.

Pollard, E.C.
The physics of viruses. New York. Academic Press. 1953.

Puck, T.T.
The first steps of virus invasion. Cold Spring Harbor Sympos. Quant. Biol. XVIII:149-154. 1953.

Puck, T.T., A. Garen, and J. Cline.
The mechanism of virus attachment to host cells. I. J. Exptl. Med. 93:65-88. 1951.

Puck, T.T. and B. Sagik.
Virus and cell interaction with ion exchangers. J. Exptl. Med. 97:807-820. 1953.

Rivers, T.M. ed.
Viral and rickettsial infections of man. 2nd. ed. Phil. Pa. J.P. Lippincott Publ. 1952.

Schlesinger, M.
Uber die bindung des bakteriophagen an homologe bakterien. Z. Hyg. u. Infectionskankh. 114:130-160. 1932.

Stahmann, M.A., L.H. Graf, J.L. Patterson, J.C. Walker, and D.W. Watson.
The inhibition of tobacco mosaic virus by synthetic lysine polypeptides. J. Biol. Chem. 189:45-52. 1951.

Stent, G.S. and E.L. Wollman.
On the two-step nature of bacteriophage adsorption. Biochem. Biophys. Acta. 8:260-269. 1952.

Swanstrom, M. and M.H. Adams.
Agar layer method for the production of high titre phage stocks. Soc. Exptl. Biol. Med. 78:372-375. 1951.

Tolmach, L.J. and T.T. Puck.
The mechanism of virus attachment to host cells. III. J. Am. Chem. Soc. 74:5551-5552. 1952.

Watson, J.D.
The properties of x-ray-inactivated bacteriophage. J. Bact. 60:697-718. 1950.

Weidel, W.
Viruses 1950. Pasadena. Cal. Tech. Publ. 1950.

APPENDIX

Production of High Titre Bacteriophage Stocks*

Bacteriophage stocks with high titres were obtained by modifications of the technique described by Swanstrom and Adams (1951). The modified method used for stock production in these experiments has proved successful with two sets of phage, the T-series of E. coli, and the Xp-series of X. pruni.

The Swanstrom Adam's procedure was followed except for the alterations described below. To petri dishes containing approximately 20 ml. of nutrient agar base was added a surface layer of 5 ml. of a semisolid nutrient agar containing host cells, and enough phage to produce complete lysis on the plate (about 1000 plaques/plate). The plates were then allowed to incubate overnight, after which the surface layers were collected in a sterile pint mason jar. The collection of the surface layer was carried out by adding 5 ml. of old stock phage suspension to each petri dish and then scrapping the surface layer off with a small sterile spatula. The collected material was then beaten in an Osterizer for 10-15 seconds and the mixture stored overnight at 12° C., and then osterized again for 15 seconds. Bacteria and remaining solid particles were removed by low speed centrifugation. The supernatant fluid was collected and assayed for phage titre.

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* In cooperation with A. Eisenstark, Dept. Bact., Kansas State College, Manhattan, Kans., and aided by a grant from the Office of Naval Research.

Titres of phage obtained by this method are compared with yields obtained with the Swanstrom and Adams technique in Table 7. .

Table 7. Comparison of phage production yields.

Phage	: Swanstrom-Adams method	: Modified method
T_2 *E. coli*	1.5×10^{12}	2.0×10^{14}
T_3 " "	2.3×10^{11}	1.2×10^{16}
T_7 " "	7.2×10^{11}	6.2×10^{14}
Xp-1 *X. pruni*	8.4×10^{7}	4.5×10^{10}
Xp-4 " "	2.8×10^{8}	3.0×10^{11}

Table 8. Raw data for typical phage experiment. Data represents time in seconds for cell to travel 32 microns.

Ratio of Phage/Bacterium						
control	1:1	5:1	50:1	100:1	200:1	500:1
2.75	3.85	3.85	6.025	6.325	4.00	4.25
3.125	4.30	3.95	6.85	6.15	4.74	3.40
2.55	4.10	3.25	6.30	6.40	4.75	5.00
3.25	3.00	3.65	6.15	6.20	3.60	4.45
2.55	3.40	3.75	6.00	6.30	2.60	3.75
2.45	3.25	4.00	6.45	5.95	3.90	4.20
2.55	2.75	3.95	5.60	6.25	4.40	4.10
3.65	3.025	3.80	6.25	6.55	5.05	4.20
2.50	2.90	3.90	6.175	6.375	3.25	5.00
2.925	3.35	3.05	6.55	5.70	5.15	4.45
2.40	3.25	3.75	5.95	5.70	3.40	5.10
3.85	2.85	3.725	5.90	5.05	5.55	4.65
2.50	2.80	3.90	5.975	5.80	4.45	4.20
2.80	3.20	3.325	5.70	5.25	4.35	5.10
3.375	3.00	3.15	6.05	6.05	5.15	4.75
2.90	3.05	4.55	6.30	6.20	5.60	4.55
2.85	3.00	3.90	6.40	5.95	5.55	3.80
3.00	3.35	3.825	6.00	5.40	5.40	3.65
2.90	3.10	4.05	6.25	6.05	4.30	4.45
3.30	2.925	3.70	5.85	5.90	4.60	4.20
2.575	3.40	3.85	5.95	5.80	4.65	5.05
3.40	3.95	3.45	5.975	5.40	5.275	3.45
2.975	3.00	3.90	5.90	5.625	5.15	4.20
4.00	3.20	3.90	5.80	5.65	5.25	4.30
3.30	3.50	4.10	6.05	5.70	5.40	3.50
3.50	3.55	4.20	6.30	5.80	5.25	3.90
2.75	3.70	3.90	5.90	5.55	4.95	3.60
3.40	3.40	4.60	5.95	5.65	5.10	3.70
3.05	3.45	4.05	5.525	5.40	5.30	3.65
2.575	3.20	3.95	5.85	5.60	5.55	4.65
2.75	2.975	3.70	5.95	5.25	5.00	4.30
3.20	3.35	3.40	5.95	5.60	4.90	3.90
2.725	3.35	3.35	5.85	5.40	4.50	4.50
3.35	3.45	3.95	6.20	5.725	5.05	4.15
3.025	3.30	3.25	6.025	6.30	6.25	4.30
2.95	3.55	4.15	6.00	6.00	4.95	4.45
3.20	3.00	3.95	5.65	5.90	5.15	3.90
2.50	3.20	4.05	6.00	5.225	5.325	4.55
3.05	3.35	4.00	6.45	6.05	4.00	3.75
3.125	3.175	3.90	6.125	6.00	5.70	4.075
3.70	2.95	4.10	6.00	5.75	6.05	4.50

Table 8. (concl.).

Ratio of Phage/Bacterium						
control	1:1	5:1	50:1	100:1	200:1	500:1
3.30	3.65	4.10	6.05	6.325	5.80	4.10
3.10	3.75	3.925	5.80	6.20	5.325	4.50
3.25	3.55	3.95	5.925	6.35	5.65	4.40
3.10	3.95	3.40	6.60	5.95	4.95	4.40
3.35	3.475	3.50	5.95	6.05	5.90	3.65
3.35	3.70	3.575	6.10	6.15	5.70	4.90
3.50	3.40	3.40	6.20	5.90	5.30	3.80
3.30	3.50	4.35	6.175	6.40	5.025	4.60
2.55	2.90	3.90	6.025	6.35	5.725	3.50
			$\bar{x}$			
3.04	3.06	3.816	6.059	5.892	4.958	4.234
			Resistance in ohms			
410	150	160	185	170	120	150
			Current (milliamps.)			
0.3	0.8	0.8	0.7	0.8	0.9	1.3
			$\bar{v}$			
9.57	9.03	7.82	4.56	4.47	4.80	4.34

$\bar{x}$ = average of the fifty time determination
$\bar{v}$ = average calculated mobility in terms of microns/sec/v/cm.

Table 9. Raw data for typical N DV strains experiment. Numbers represent time in seconds for cells to travel 100 microns, and are the average of 5 single cell observations in one direction.

Roakin - Dilution of Virus							
control		1:50		1:5		1:1	
1.75	2.75	2.50	3.40	2.65	6.05	7.00	5.60
1.60	2.65	3.35	2.35	3.10	4.60	8.35	3.40
1.85	3.00	4.10	2.60	3.90	5.10	6.10	4.85
1.75	2.90	2.00	3.30	2.30	3.80	7.65	5.15
1.55	2.70	2.30	3.10	2.15	4.35	7.25	3.90
$\bar{x}$							
1.70	2.80	2.83	2.95	2.82	4.78	7.27	4.56
$\bar{\bar{x}}$							
2.25		2.89		3.80		5.92	
Resistance in ohms							
50		42		38		25	
Current (milliamps.)							
7.0		7.0		7.0		7.0	
$\bar{v}$							
14.18		13.85		11.18		10.80	

$\bar{x}$ = average of each of the eight grouped readings
$\bar{\bar{x}}$ = average of the $\bar{x}$ for each dilution
$\bar{v}$ = mobility in terms of microns/sec/v/cm.

ELECTROPHORETIC CHANGES IN CELL SURFACES DUE TO ATTACHMENT OF VIRUS PARTICLES

by

LEWIS B. BERNSTEIN

A.B., Kenyon College, 1952

ABSTRACT OF A THESIS

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OF AGRICULTURE AND APPLIED SCIENCE

1954

Microelectrophoresis has been used as a tool for studying bacterial surface properties for over twenty years. Standardization of the procedures for microelectrophoretic investigation has now made the technique applicable for routine laboratory use. None-the-less, a general consideration of the method as a means of definitively characterizing a virus or members of a virus series, such as the T-series of Escherichia coli, has been overlooked. This thesis was designed to fill in this gap.

Investigations were limited to consideration of two virus series; Newcastle Disease Virus (NDV) strains Roakin, Manhattan, and California, and the Xp bacteriophage series for Xanthomonas pruni. Three phases of study were pursued with each system; (1) differences in electrophoretic mobilities (EPMs) of cells in contact with the various virus strains or phage types, (2) differences in EPM upon addition of the virus or phage in contact with the cells, and (3) determination of the saturation point of the host cells for its virus or phage. With the Xp-series an additional phase was examined, the separation of a mixed population of phage-infected and non-infected host cells on the basis of EPM.

The method described by Moyer (1936) for using the Northrop-Kunitz electrophoresis cell was followed. Bacterial cells were washed three times and resuspended in 0.01 m sodium acetate buffer (pH 7.05), and the chick red blood cells were washed and suspended in 1/15 m phosphate buffer before intro-

duction into the electrophoretic cell. Appropriate dilution of the virus or phage with their respective host cells resulted in the desired known ratios of virus to cells.

Differential decreasing rates of mobility were observed to occur when varying ratios of X. pruni phage strains to susceptible X. pruni host cells were mixed, and when different concentrations of NDV strains were added to red blood cells. The total per cent decrease in EPM caused by each strain was found to be characteristic of the strain in both X. pruni phage system and the NDV system. An estimate of the maximum numbers of phage particles which can be adsorbed to susceptible X. pruni host cells was found to be 200 phage particles per bacterium. The total decrease in EPM of host cells caused by the addition of Xp phage closely paralleled the serological groupings of the phage.

Three strains of X. pruni differing in their susceptibility to phage action were found to have different EPMs. It was found possible to quantitatively detect the numbers of phage-infected from non-infected cells in a mixed population on the basis of their different EPMs. No change in EPM of X. pruni cells resistant to phage action was found to occur upon addition of Xp phage. Red blood cells which had previously been treated with NDV, and the virus then eluted and removed, also showed no change in EPM upon the addition of fresh virus of the same strain. The most infective of the three strains of NDV tested showed a significantly larger percentage decrease in

the EPM of chicken erythrocytes than did the other two strains.

The results of these experiments further substantiate the current concept that the primary nature of virus-host attachment is electrostatic. That the ability of viruses to attach to cell surfaces is, for the most part, governed by surface charges was clearly demonstrated. But, if surface charges were the only factors operating in attachment of virus and host cell, the EPM of the host cell would logically be expected to approach zero with the addition of sufficient virus to completely saturate the cell charge. It was found, however, that only a partial reduction in total EPM occurred no matter how many virus or phage particles were added, and that the residual mobility was well above zero. The total number of virus particles that were found to attach to each cell would barely cover one two-hundreth of the total cell surface (in the case of X. pruni), and therefore occludes the possibility of the mass of phage particles per bacterium being large enough to block other phage particles from being adsorbed. This leads to the inevitable conclusion that the electrostatic sites where attachment takes place must have a specific configuration. The fact that host-range mutants specifically resistant to phage action showed no change in EPM supplements this conclusion, and would also indicate that the fundamental control of phage attachment rests in a genetic mechanism.

The parallelism between the Xp-phage serological relationships and the total decrease in EPM that they are able to

cause, and characteristic differences in EPM found for three strains of X. pruni which differed only in their capacities to adsorb phage, again points to a relationship between surface charge configuration and genotypic constitution of the "organisms". Practical application could conceivably be made of this as a rapid preliminary screening procedure for determining serological placement for new members of an established phage series.

The ability to distinguish virus-infected from non-infected cells in a mixed population of the two, can be made use of qualitatively and/or quantitatively as a means of checking suspect infected or lysogenic cultures.