VIRUS PARTICLE ADSORPTION

II. ADSORPTION OF VACCINIA AND FOWL PLAGUE VIRUSES TO CELLS IN SUSPENSION

A. C. ALLISON AND R. C. VALENTINE
National Institute for Medical Research, Mill Hill, London (England)

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SUMMARY

The rates of adsorption of radioactively-labelled fowl plague and vaccinia viruses to fowl red blood cells, chick embryo cells, HeLa cells and ascites tumour cells in suspension have been studied. The observed rates were less than the calculated rates of collision between virus particles and cells. The initial rates of adsorption corresponded to those expected if one third of collisions between virus particles and cells is effective for adsorption. Later the rates of adsorption fell, mainly owing to clumping of the cells. The rates of adsorption were the same to cells which support virus multiplication and those which do not. Elution of fowl plague virus from fowl red blood cells at 37° was rapid, but very little elution of fowl plague or vaccinia viruses took place from tissue culture cells at 37°.

INTRODUCTION

In the first paper of this series[1] equations were derived to define the rate of collision between small particles, such as viruses, and larger spherical objects, such as cells, suspended in a fluid medium. Reasons were given why the approximate forms of the equations that can be used to calculate the collision rates between phage particles and host bacteria are not applicable to animal viruses and their host cells.

In this paper the adsorption of radioactively labelled viruses to a variety of host cells is described. Some of the host cells used were capable of supporting the multiplication of the viruses, others were not. In all cases, however, the rates of attachment of virus particles to cells were of the same order, which was several times less than that expected from Brownian theory. This stands in contrast to the rates of attachment of viruses to glass and other non-biological surfaces which under optimal conditions are very close to those expected from Brownian theory[1]. In a later paper[2] the rates of attachment of viruses to cell monolayers will be described, and the effects of some variables on adsorption analysed. These results suggest reasons why the adsorption of viruses to cells is less efficient than adsorption to aluminium, glass, nitrocellulose and carbon surfaces. They are also of practical use in calculating the proportion of virus particles adsorbed in experiments on virus multiplication.

MATERIALS AND METHODS

Virus

The preparation of purified ^{131}I-labelled vaccinia virus and ^{32}P-labelled fowl plague virus, and techniques used for assaying radioactivity, have been described previously[1].

Red blood cells

The red blood cells used were obtained from adult fowl blood collected in acid–citrate–dextrose, centrifuged, the buffy coat removed and the cells washed twice in normal saline. The cells used were not agglutinated by vaccinia.

Ascites tumour cells

S 37 sarcoma ascites tumour cells were passaged at 10 day intervals in C3H mice. For experimental purposes they were harvested after 8 days, washed twice in Gey's solution and resuspended by vigorous pipetting. They were allowed to stand for 1 min in a siliconized tube and the uppermost cells used.

HeLa cells

HeLa cells were propagated in the following growth medium: 70 parts of Gey's solution; 10 parts of 5% lactalbumin hydrolysate; 10 parts of 1% yeast extract; 10 parts of human serum. Cultures were washed with calcium- and magnesium-free saline (Gey's solution A) and incubated for 15 min at 37° in phosphate buffered saline containing 0.05% trypsin. The liberated cells were washed twice in Gey's solution and resuspended by vigorous pipetting in the adsorbing medium. They were allowed to stand for 1 min in a siliconized tube and the uppermost layer of cells were used.

Chick embryo cells

Petri dish cultures of cells from 10 day old chick embryos were prepared by a modification[3] of DULBECCO's technique. The cultures were incubated at 37° in growth medium until a confluent layer of cells was obtained. The cultures were washed, trypsinized and again washed and resuspended as described for the HeLa cells.

Cell counts and measurements

Cell counts were made in a haemocytometer, 1000 cells being counted in each case, which reduces the random error of the count to less than 4%. Cell diameters were measured in wet suspensions by a Filar micrometer and by measurement of photomicrographs. Agreement of the results by the two techniques was satisfactory. With each cell type distributions of radii were plotted and the geometric mean was taken for calculations.

THEORETICAL

It has been shown[1] that the maximum fraction of virus particles that can be adsorbed by a suspension of cells, *i.e.* when every collision between a particle and a cell leads to adsorption, is given by

$$f = 1 - \exp\{-4\pi nRD(t + R\sqrt{t/\pi D})\} \qquad (1)$$

where n is the number of cells of radius R, t is the time and D is the diffusion coefficient of the virus. D can be calculated from the STOKES-EINSTEIN equation,

$$D = \frac{kT}{6\pi\eta a} \qquad (2)$$

where k is BOLTZMANN's constant, T the absolute temperature, η the viscosity of the suspending fluid and a the radius of the particles.

The experiments to be reported below show that the observed rate of adsorption of virus is considerably less than that expected from equation (1). When only a fraction of the collisions is successful (*i.e.* leads to adsorption), an exact mathematical treatment of this situation is excessively difficult. In the early stages of adsorption, however, the form of the equation will be similar to the above, but with a factor ϕ equal to the proportion of successful collisions multiplying the exponential term, *i.e.*

$$f = 1 - \exp\{-4\phi\pi nRD(t + R\sqrt{t/\pi D})\} \qquad (3)$$

Where the proportion of successful collisions is very small (small values of ϕ), this equation will be very approximate except for a short period after commencing adsorption. However, if $\phi \geqslant 1/3$, as we have found it to be in most cases of animal virus adsorption, equation (3) appears to be closely followed for periods at least up to 30 min, when other factors, notably clumping of cells, significantly alter the adsorption rate.

EXPERIMENTAL

Eight tubes containing 1.9 ml washed red cells in Gey's solution without bicarbonate, buffered to pH 7.0 with 0.02 M sodium phosphate were prepared, four with 10^7 cells/ml and four with $5 \cdot 10^6$ cells/ml. At zero time 0.1 ml ^{32}P-labelled fowl plague virus was added to the cell suspension, which was resuspended by shaking every 7.5 min. At 7.5 min, 15 min, 30 min and 60 min the cells were chilled and centrifuged, the supernatant removed and the cells washed twice in cold Gey's solution. Samples of the supernatant, and volumes of the resuspended red cells, were dried down on 1 cm^2 planchettes for assay of radioactivity in an end-window counter. The proportion of the original count associated with the cell fraction was taken as the proportion of virus adsorbed after various time intervals. In most experiments this result was 4 to 7% lower than the count expected by subtraction of the final count in the supernatant from the original count. This small discrepancy was due partly to loss of virus during washing, and partly to elution of virus from the cells; this takes place to some extent even in the cold (see below).

The results are shown in Fig. 1. Curve A shows the maximum rate of adsorption calculated from equation (1) on the assumption that every collision between virus particles and cells results in adsorption. Curve B is calculated on the assumption that the fraction of effective collisions is one third. The observed rate of attachment of virus to cells is given in curve C, which initially coincides with curve B. When about 60% of the virus is adsorbed, however, the observed rate of adsorption falls. That this was not due to the presence of a proportion of virus particles less easily adsorbed than the rest was shown in experiments in which the virus remaining in the supernatant after 15 min was added to another suspension of cells. The rate of adsorption was then similar to that initially observed. An important factor con-

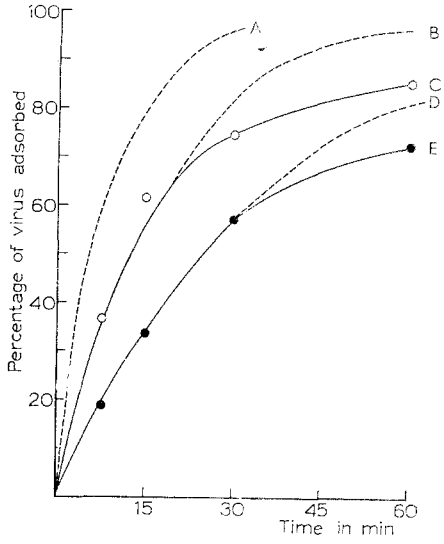

Fig. 1. Adsorption of labelled fowl plague virus by fowl red blood cells in suspension. A – theoretical maximum adsorption rate for 10^7 cells/ml. B – adsorption rate calculated for 10^7 cells/ml assuming one third of collisions are effective. C – observed adsorption rate by 10 cells/ml. D – the adsorption rate calculated for $5 \cdot 10^6$ cells with one third of collisions effective. E – observed adsorption rate for $5 \cdot 10^6$ cells.

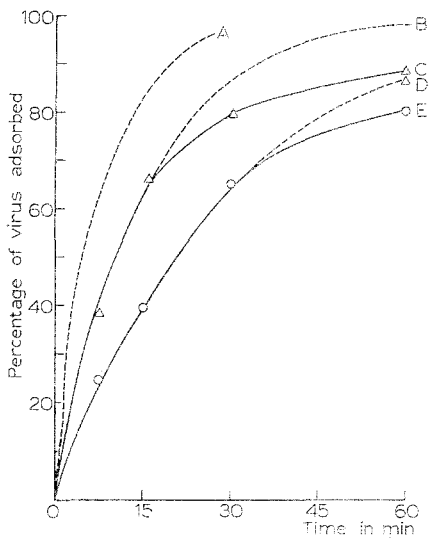

Fig. 2. Adsorption of labelled fowl plague virus by chick embryo cells in suspension. A – theoretical maximum adsorption rate for 10^7 cells/ml. B – adsorption rate calculated for 10^7 cells/ml assuming one third of collisions are effective. C – observed adsorption rate by 10^7 cells/ml. D – the adsorption rate calculated for $5 \cdot 10^6$ cells with one third of collisions effective. E – observed adsorption rate for $5 \cdot 10^6$ cells.

tributing to the fall in adsorption rate is clumping of the cells; this was evident microscopically after about 15 min under these conditions. This reduces the cell area available for virus attachment. The fact that some elution of virus occurs even at 20° (see below) could also be contributory.

Adsorption of fowl plague virus by suspensions of chick embryo cells and HeLa cells

Suspensions of 10^7 and $5 \cdot 10^6$ chick embryo cells/ml were prepared in buffered Gey's solution as described in the section on METHODS. The rate of adsorption of ^{32}P-labelled fowl plague virus was measured as for red cells. The results are shown in Fig. 2. The initial adsorption rate again coincides with that expected on the assumption that one third of the surface area is effective. The rate of adsorption by HeLa cells, in which this strain of fowl plague grows poorly, was also of the same order of magnitude (Fig. 3).

Adsorption of vaccinia virus by HeLa cell suspensions

The rate of adsorption of vaccinia virus to HeLa cell suspensions is also shown in Fig. 3. The rate is lower than that of fowl plague virus, by the factor expected from the difference in diffusion coefficients of the two viruses. Again, the initial adsorption rate coincided with the expectation from equation 1 on the assumption that one third of the collisions are effective.

Adsorption of vaccinia virus by ascites tumour cells in suspension

The rate of adsorption of labelled vaccinia virus to ascites tumour cells in sus-

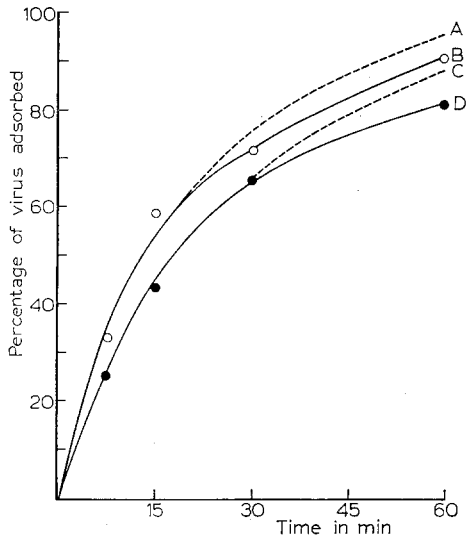

Fig. 3. Adsorption of labelled fowl plague and vaccinia viruses by HeLa cells in suspension A – adsorption rate calculated for fowl plague virus by $5 \cdot 10^6$ HeLa cells/ml on the assumption that one third of collisions are effective. B – observed adsorption rate of fowl plague virus by $5 \cdot 10^6$ HeLa cells/ml. C – adsorption rate calculated for vaccinia virus by $5 \cdot 10^6$ HeLa cells in suspension assuming that one third of collisions are effective. D – observed adsorption rate of vaccinia virus by $5 \cdot 10^6$ HeLa cells/ml.

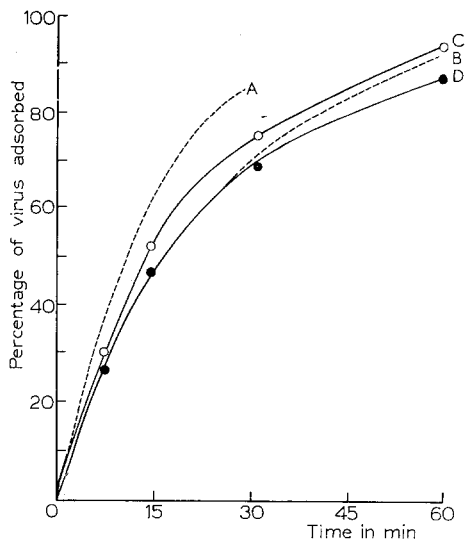

Fig. 4. Adsorption of labelled vaccinia virus by ascites tumour cells in suspension. A – adsorption rate calculated for vaccinia virus by $5 \cdot 10^6$ ascites tumour cells/ml assuming one half of collisions are effective. B – the same assuming that one third of collisions are effective. C – observed rate of adsorption of vaccinia virus by $5 \cdot 10^6$ ascites tumour cells/ml. D – observed rate of adsorption of vaccinia virus by $5 \cdot 10^6$ trypsin-treated ascites tumour cells/ml.

pension is shown in Fig. 4. The rate was actually somewhat higher than that expected on the assumption that one-third of the collisions with the cells result in adsorption, but it fell to almost exactly this figure after treatment of the cells with trypsin.

Elution of virus from cells

The elution of labelled fowl plague virus from chick embryo cells, HeLa cells and fowl red blood cells was investigated. Cells to which virus had been adsorbed at room temperature were washed in cold Gey's solution and resuspended in Gey's solution without bicarbonate buffered with 0.02 *M* sodium phosphate at pH 7.0. Large volumes were used to reduce readsorption of eluted virus by cells. After 1 h at 4°, 20° and 37° the cells were centrifuged and radioactivity (representing virus still associated with cells) was counted. In Table I the proportion of virus eluted from

TABLE I

PERCENTAGE OF ^{32}P-LABELLED FOWL PLAGUE VIRUS ORIGINALLY ATTACHED TO CELLS ELUTED AT VARIOUS TEMPERATURES (pH 7.0)

Temperature	*0°*	*20°*	*37°*
Fowl red cells	8	20	95
Chick embryo cells	4	4	7

the cells under these conditions is shown. As expected, at 37° nearly all the virus adsorbed by red cells was eluted, but some elution occurred even at 4°. With chick embryo cells, the rate of elution at all temperatures was low. This was found to be true also of vaccinia virus attached to chick embryo cells.

DISCUSSION

The rate of adsorption of bacteriophage particles to bacteria suspended in a fluid medium has often been expressed[4–7] in terms of a rate constant K defined by the equation

$$f = 1 - \exp(-Knt)$$

where f is the fraction of particles n adsorbed by bacilli at time t after mixing. The rate constant K can be calculated from

$$K = 4\pi RD$$

where R is the radius of the adsorbing cells and D is the diffusion coefficient of the virus. PUCK has shown that the transient term, which appears in the VON SMOLUCHOWSKI formulation, can be neglected for all practical purposes in this system.

Attempts have, however, been made to use the same equations to describe the adsorption of animal viruses to suspensions of red blood cells[9] and tissue culture cells[9–11] which have radii of 4 to 10 μ. As previously pointed out[1], in these systems K will not be a constant until about 1 h after adsorption starts. A more exact formulation is given in equation (3).

The main finding in our experiments has been that the two dissimilar animal viruses studied, vaccinia and fowl plague, are adsorbed by cells in suspension at rates considerably below those expected from the calculated rates of collision between virus particles and cells. Initially, adsorption occurs at rates which suggest that one third of the collisions between virus particles and cells are effective for adsorption. Later the rates of adsorption fall, mainly because of clumping of the cells, which reduces their effective surface area.

It seems clear that the efficiency of virus adsorption relative to collision frequency is somewhat higher in monolayers of cells (one half of collisions effective[2]) than in trypsinized suspensions of the same cells (one third of collisions effective). This suggests that trypsinization removes from the cell surface a protein component which not only promotes intercellular adhesion, but also facilitates virus adsorption, perhaps because it alters the charge of the cell surface. There is evidence that trypsinization of S 37 ascites tumour cells produces a significant loss of dry mass measured by interferometry[12], and enzyme treatment of ascites tumour cells changes their electrophoretic mobility[13]. Measurements of electrophoretic mobility of red cells show differences from white cells[14], and it is interesting that, so far as efficiency of virus adsorption is concerned, untreated red cells should behave like trypsinized tissue culture cells.

However, an obvious difference between red cells and tissue cultures shows up in elution. Fowl plague virus is rapidly eluted from red cells at 37°, but only slowly from chick embryo cells, in accordance with previous observations on Newcastle disease[9]. The quantitative studies of adsorption here reported are useful from the practical point of view, since they enable reasonably accurate calculations to be made

of the proportion of virus adsorbed by cells in experiments on virus multiplication. The results of other workers with herpes B[15], poliovirus[11] and Newcastle disease[9,10] suggest that the same order of efficiency is attained with other viruses. Although the efficiency of adsorption is lower, adsorption of virus particles by suspended cells is much more rapid than adsorption from similar volumes by cell monolayers. This is not only true because the surface area of the cells is greater in suspension, but there is also a smaller distance over which virus particles have to diffuse before reaching cell surfaces. This expectation is borne out in practice, despite statements to the contrary[9].

It is of interest that the rates of adsorption of vaccinia and fowl plague were of the same order in cells in which the viruses multiply readily and those in which they do not. Thus the strain of fowl plague used multiplies rapidly in embryo cells but not in HeLa cells; the strain of vaccinia used multiplies rapidly in HeLa cells but not in ascites tumour cells. Thus the barrier to multiplication in these cases does not arise from a failure of the virus to adsorb to the cells. This finding contrasts with the behaviour of poliovirus, which is reported to adsorb readily only to susceptible cells[16]. There may be various factors involved in cellular resistance to virus infection.

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