VIRUS PARTICLE ADSORPTION

I. THEORY OF ADSORPTION AND EXPERIMENTS ON THE ATTACHMENT OF PARTICLES TO NON-BIOLOGICAL SURFACES

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SUMMARY

It has been shown experimentally that even in a shaken system the rate at which virus and similarly sized particles in suspension arrive at a surface can be accurately predicted from the theory of Brownian motion. Equations are deduced from which the rate of arrival of particles on flat and spherical surfaces can be calculated. Experiments with various non-biological surfaces have shown that all the impinging particles are adsorbed when a sufficient concentration of ions is present. The effects of various concentrations of different salts on rates of particle adsorption have been investigated. It is concluded that the results are at variance with current theories of particle-surface interactions. They also suggest that a considerable delay in the start of a virus infection may result from the long time needed for the virus to reach a cell surface.

INTRODUCTION

Virus particles produce a biological effect only after adsorption to a cell surface, and for this to occur the virus obviously has to be able to reach the surface and interact with it. Viruses are not actively motile and must rely for transport on their own random Brownian motion and on movements of the fluid in which they are suspended.

The experiments to be described here were an attempt to find out the factors determining the rate at which particles in a suspending fluid reach a surface and what then determines whether they will be adsorbed to it. Of particular interest was the question of whether the adsorption of a virus particle to a cell surface differs in any significant way from the adsorption of a non-biological object of a similar size to an inorganic surface. The first part of this paper is concerned with the theory of Brownian motion as it relates to the rate at which particles in suspension will be expected to arrive at a surface. The second part is a description of experiments to determine the rate at which virus particles and polystyrene latex spheres are actually adsorbed by various non-biological surfaces. The counts of the numbers of adsorbed particles were made either with the electron microscope or by using radioactively labelled particles. In a subsequent paper these basic observations will be extended to the adsorption of viruses to cell monolayers and to cells in suspension.

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THEORY

Summary of symbols used

a, radius of particle
A, adsorbing area
c, concentration of particles in the fluid (number per unit volume)
c_0, concentration of particles at the start
d, depth of fluid above surface
D, diffusion constant of particles
e, base of natural logarithms (2.718)
f, fraction of the initially suspended particles adsorbed
k, Boltzmann's constant ($1.38 \cdot 10^{-16}$)
K, rate constant
m, an integer
n, number of adsorbing spheres per unit volume
N, number of particles adsorbed per unit surface area
P, number of unadsorbed particles per unit volume
r, distance from centre of adsorbing sphere
R, radius of adsorbing sphere
t, time
T, absolute temperature (°C + 273)
x, space co-ordinate normal to adsorbing surface
β, constant of integration
η, viscosity of suspending fluid

We consider first the problem of a flat surface covered to a depth d with a suspension of uniform spherical particles moving with a diffusion constant D. An expression is derived for the number of particles that collide with the surface in a time t. This expression gives the maximum rate at which particles can be adsorbed on to the surface, *i.e.* when every particle that collides with it remains permanently attached. Motion of the fluid and sedimentation of the particles are ignored as it will be shown experimentally that in most cases they have a negligible effect on the result.

Particles in random motion conform to the diffusion equation which, if the x-coordinate is taken in the direction normal to the surface, has the form

$$\frac{dc}{dt} = D \frac{\partial^2 c}{\partial x^2} \tag{1}$$

where c is the concentration of particles at any point.

For spherical particles moving in Brownian motion, the diffusion constant D is given by[1]

$$D = \frac{kT}{6\pi\eta a} \tag{2}$$

where k is Boltzmann's constant ($1.38 \cdot 10^{-16}$), T the absolute temperature, η the viscosity of the suspending fluid and a the radius of the particles. If, in addition to Brownian motion, other factors are acting but the motion remains random, then the same equation (1) will hold but D will be larger than the value given by equation (2).

Initially the particles are uniformly suspended with a concentration everywhere

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of $c = c_0$. Adsorption starts and if we assume that every collision with the surface leads to a permanent attachment of the particle, then at the surface $c = 0$ since the adsorbed particles are no longer free to diffuse. A concentration gradient is thus set up and the rate at which particles hit a unit area of surface is given (from the definition of the diffusion constant) by

$$\frac{dN}{dt} = D\left(\frac{\partial c}{\partial x}\right)_{x=0} \tag{3}$$

where $\left(\frac{\partial c}{\partial x}\right)_{x=0}$ is to be obtained by solving the diffusion equation (1) for the appropriate boundary conditions. We already have for these

$$t = 0 \quad c = c_0 \text{ for } 0 < x < d \tag{4}$$

$$t > 0 \quad c = 0 \text{ for } x = 0 \tag{5}$$

and a third condition depends on whether the particles are adsorbed or reflected at the top surface of the fluid. In the former case, for $t > 0$ $c = 0$ for $x = d$ while in the latter we introduce an artificial "mirror image" fluid above the top surface with $c = 0$ again only when $x = 2d$. Since we shall be concerned mainly with cases where adsorption on the top surface is prevented and since in any case the difference only affects the numbers adsorbed to the surface after long times, the second condition will be taken to apply and we shall have

$$t > 0 \quad c = 0 \text{ for } x = 2d \tag{6}$$

The solution of the diffusion equation (1) with the boundary conditions (4), (5) and (6) is given by[2]

$$c = \sum_{m=0}^{m=\infty} \frac{4c_0}{(2m+1)\pi} \sin\left[\frac{(2m+1)\pi x}{2d}\right] e^{-(2m+1)^2\pi^2 Dt/4d^2}$$

$$\therefore \frac{\partial c}{\partial x} = \sum_{m=0}^{m=\infty} \frac{2c_0}{d} \cos\left[\frac{(2m+1)\pi x}{2d}\right] e^{-(2m+1)^2\pi^2 Dt/4d^2}$$

and $$\left(\frac{\partial c}{\partial x}\right)_{x=0} = \sum_{m=0}^{m=\infty} \frac{2c_0}{d} e^{-(2m+1)^2\pi^2 Dt/4d^2}$$

On substituting this back into equation (3) we obtain

$$\frac{dN}{dt} = \frac{2Dc_0}{d} \sum_{m=0}^{m=\infty} e^{-(2m+1)^2\pi^2 Dt/4d^2}$$

and on integration this gives the number of particles adsorbed on unit area after a time t as

$$N = \beta - \frac{8dc_0}{\pi^2}\left[\sum_{m=0}^{m=\infty} \frac{1}{(2m+1)^2} e^{-(2m+1)^2\pi^2 Dt/4d^2}\right]$$

where β is a constant of integration. Now as t becomes large, the exponential term tends to zero so that β is, in fact, the number of adsorbed particles per unit area when $t = \infty$, *i.e.* the number originally in suspension above unit area. Thus $\beta = c_0 d$ and so the fraction f of the particles originally in suspension which are adsorbed to

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a flat surface after a time t (assuming that every collision between a particle and the surface leads to adsorption) is given by

$$f = \frac{N}{\beta} = 1 - \frac{8}{\pi^2} \sum_{m=0}^{m=\infty} \frac{1}{(2m+1)^2} e^{-(2m+1)^2 \pi^2 Dt/4d^2}.$$

The infinite series can be replaced by one of two approximations (accurate to better than 1%) depending on the value of Dt/d^2. First, for $Dt/d^2 = 0$ to 0.12,

$$f = \frac{1.13\sqrt{Dt}}{d}. \tag{7}$$

This expression holds until 40% of the particles have been adsorbed on the surface. For $Dt/d^2 = 0.06$ to ∞

$$f = 1 - \frac{8}{\pi^2} e^{-\pi^2 Dt/4d^2} \tag{8}$$

and this approximation can be used when more than 28% of the particles have been adsorbed.

In terms of the actual number N of particles adsorbed per unit area these expressions become: for less than 40% of the particles adsorbed

$$N = 1.13\, c_0 \sqrt{Dt}, \tag{9}$$

for more than 28% of the particles adsorbed

$$N = c_0 d \left(1 - \frac{8}{\pi} e^{-\pi^2 Dt/4d^2}\right). \tag{10}$$

For particles in Brownian motion, the value of the diffusion constant D to be used in equations (7) to (10) is given by equation (2).

We now derive an expression for the maximum rate at which particles can be adsorbed by a suspension of cells, *i.e.* when every collision between a particle and a cell leads to permanent adsorption. The cells are assumed to be uniform spheres of radius R and the effects of sedimentation and convection again ignored.

First we consider the case of a single sphere which adsorbs particles from an effectively infinite volume of fluid containing initially c_0 particles in unit volume. When adsorption starts the concentration at the cell surface falls to zero (since the adsorbed particles are no longer free to diffuse) and a concentration gradient is set up. After a time interval t the concentration c of particles at any distance r from the centre of the sphere will be given by[3]

$$c_0 - c = \frac{Rc_0}{r}\left(1 - \operatorname{erf}\frac{r-R}{2\sqrt{Dt}}\right) \tag{11}$$

where the diffusion constant D of the particles, if they are spherical and move in Brownian motion only, is given by equation (2). Erf stands for error function (*i.e.* $\operatorname{erf} x = \frac{2}{\sqrt{\pi}} \int_0^x e^{-z^2} dz$).

On differentiating equation (11) along a normal to the surface of the adsorbing sphere and using the expression

$$\frac{d}{dr}\left(\operatorname{erf}\frac{r-R}{2\sqrt{Dt}}\right) = -\frac{1}{\sqrt{\pi Dt}} e^{-(r-R)^2/4Dt},$$

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we obtain

$$\frac{dc}{dr} = \frac{Rc_0}{r^2}\left(1 - \operatorname{erf}\frac{r-R}{2\sqrt{Dt}}\right) + \frac{Rc_0}{r\sqrt{\pi Dt}}\,e^{-(r-R)^2/4Dt}. \qquad (12)$$

Now the rate of arrival of the particles on the surface of the sphere is given, by the definition of D, as

$$-\frac{dc}{dt} = 4\pi R^2 D\left(\frac{dc}{dr}\right)_{r=R}$$

i.e., using equation (12) after substituting $r = R$,

$$-\frac{dc}{dt} = 4\pi R^2 D c_0\left(\frac{1}{R} + \frac{1}{\sqrt{\pi Dt}}\right). \qquad (13)$$

Suppose now that we have not an infinite volume of fluid but one whose volume is nevertheless considerably larger than that of the adsorbing sphere. Except near the surface of the sphere, we shall have a nearly uniform but slowly falling concentration c of particles and the rate of adsorption will thus be very nearly given by substituting c for c_0 in equation (13)

$$-\frac{dc}{dt} = 4\pi R^2 D c\left(\frac{1}{R} + \frac{1}{\sqrt{\pi Dt}}\right).$$

If now then there are not one but n similar adsorbing spheres in unit volume, we can write, after separating the variables,

$$\frac{dc}{c} = -4\pi nRD\left(1 + \frac{R}{\sqrt{\pi Dt}}\right)dt$$

and after integration

$$c = c_0\, e^{-4\pi n RD\,(t\, +\, R\sqrt{t/\pi D})}.$$

The fraction of the original number of particles adsorbed on the cells after a time t is thus finally obtained

$$f = \frac{c_0 - c}{c_0} = 1 - e^{-4\pi n RD\,(t\, +\, R\sqrt{t/\pi D})}. \qquad (14)$$

The rate of adsorption of particles to cells has often been expressed in terms of a rate constant K defined by the equation

$$-\frac{dP}{dt} = KPn$$

$$i.e.\ P = P_0\, e^{-Knt}$$

$$\text{or } f = 1 - e^{-Knt} \qquad (15)$$

where P is the number of unattached particles per unit volume at any time t and n the number of adsorbing cells. This use of a rate constant was introduced some time ago as a convenient way of expressing rates of phage adsorption by bacteria[4–6]. However, equation (14) shows that in fact K so defined will only be a constant when the second term in the exponential $R\sqrt{t/\pi D}$ is negligible compared with t. This requires that R is very much smaller than $\sqrt{\pi Dt}$. Now for viruses, D is of the order of 10^{-8} so that after, say, 60 sec, $\sqrt{\pi Dt}$ is about 14 μ. Thus for the adsorption of phage to bacteria, which have a radius of about 0.5 μ, K is a true constant and is a useful

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measure of the rate of adsorption. In such cases $K = 4\pi RD$ as can be seen by comparing equations (14) and (15). But equation (15) has also been used for adsorption of viruses to red blood cells[7] and to tissue culture cells[8,9] which have radii of 4–10 μ and here K should not be a constant until about an hour after adsorption starts. With these larger cells, therefore, the use of a rate constant may not be a satisfactory way of expressing rates of adsorption. For adsorption on to flat surfaces and cell monolayers the use of a rate constant K is certainly quite unjustifiable. As equations (7) and (8) show, for a flat surface it is only in the later stages of adsorption that an equation of the form of (15) is approximately valid and even then any measurement of a rate constant would depend entirely on the depth of fluid used.

EXPERIMENTAL

Preparation of the adsorbing surfaces

Electron microscope specimen supports were prepared in the usual way with a film of nitrocellulose. In some experiments this film was then covered with a layer of carbon by placing the supports 10 cm below a pair of carbon rods passing 50 A in a high vacuum for 2 sec[10]. In other cases the films were covered with a layer of aluminium or gold by evaporating on to them a small length of wire hung from a tungsten filament in a high vacuum as for the usual metal-shadowing technique of electron microscopy.

Glass coverslips were also used as adsorbing surfaces. These were well cleaned and then broken into rectangular pieces measuring 2 cm × 1 cm. For some of the experiments a layer of aluminium was similarly deposited on them.

Polystyrene latex particles

Polystyrene latex spheres are available in distilled water suspensions containing particles of a uniform size[11]. (These suspensions are available through the generosity of the Dow Chemical Corporation, Midland, Michigan, U.S.A.) The particles can be obtained as monodispersed suspensions covering a range of sizes between 0.08 μ and 1.2 μ in diameter and the following batches were used: LS-040-A; LS-055-A; LS-066-A; 15N-8; 580-G (the latter is no longer obtainable).

The diameter of the particles in batch 580-G has been accurately determined in a number of laboratories and a figure of 0.259 μ agreed on. The diameters of the particles in the other batches were measured by mixing them with 580 G and measuring the ratio of the diameters on electron micrographs. The results were confirmed by measurements made on electron micrographs of the particles on aluminium replicas of a standard grating; in this case the ratio of particle diameters to the space between the grating rulings was found. The mean diameters obtained were: LS-040-A, 0.08μ; LS-055-A, 0.16μ; 15N-8, 0.46μ; LS-066-A, 0.69μ. In some cases these figures are appreciably (up to 15%) lower than those given by the manufacturers (*cf.* KELLENBERGER's remarks[12]).

Particle counts in the suspensions were determined from the dry weights of 0.1 ml volumes of the suspensions as supplied which contained about 10% w/w of solids. A figure of 1.05 g/ml was taken for the density of the latex[12,13]. The calibrated suspensions were then accurately diluted with a suitable volume of water before use.

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[^{32}P]-labelled fowl plague virus

WECKER AND SCHÄFER[14] and FRANKLIN, RUBIN and DAVIS[15] have reported the incorporation of ^{32}P into fowl plague and Newcastle disease viruses grown in tissue culture with very much higher activities than those obtained by other workers growing viruses in eggs. The technique described here has given the highest specific labelling so far reported for an animal virus.

Six 10-day-old chick embryos were treated with trypsin, washed[16] and resuspended in 150 ml of the following medium: 2.5 ml calf serum, 0.5 ml lactalbumin hydrolysate, 100 units penicillin, 100 μg streptomycin and 50 units Mycostatin per 100 ml Gey's solution. 15 ml carrier-free ^{32}P (3 mC) was added to the suspension, which was distributed in 3 1-litre Roux flasks and incubated overnight. Fowl plague virus (Dutch strain) was added to the suspensions to give $2.3 \cdot 10^3$ plaque-forming units per ml. The virus was harvested after 3 days' incubation at 37°.

The fluid was decanted and cooled to 1° and the virus adsorbed on to fowl red cells. The tissue cells left in the culture flasks were suspended in Gey's solution without bicarbonate and frozen and thawed twice. The liberated virus was similarly adsorbed on to red cells and all the red cells were then pooled and well washed with ice-cold saline. The virus was eluted from them by incubating at 37° for 30 min. After sedimenting the cells, the supernatant containing the eluted virus was dialysed overnight and the virus particles deposited by centrifuging at 20,000 *g* for 2 h. The deposit was resuspended and the final product had a virus particle count (determined by electron microscopy) of $8.4 \cdot 10^{10}$ particles per ml and a specific activity of 1060 counts/min per haemagglutinating dose.

[^{131}I]-labelled vaccinia virus

A suspension of vaccinia virus was obtained from rabbits and concentrated and partly purified by differential centrifugation. The virus was then flocculated in 1 *M* NaCl[17], washed and the highly purified product was suspended in 0.004 *M* phosphate–citrate buffer, pH 6.5. 2.3 ml (1.8 mg virus) received[18] 0.30 mg ^{131}I + carrier-free iodine dropwise until the solution had a faint yellow colour followed by 0.15 ml 0.25 *M* glycine buffer, pH 9.5. This decolourised the solution, which was then dialysed to remove all the radioactivity not bound to the virus. Of 205 μC added initially, 34 μC remained in the virus suspension.

Adsorption

The latex particles were adsorbed on to the films covering the electron microscope specimen supports by placing drops of about 2 mm diameter of a suspension containing 10^{10}–10^{11} particles per ml on each film. The preparations were left in a water-saturated atmosphere for various times up to 30 min and then the supports were washed in a large volume of distilled water to remove the unadsorbed particles.

Latex particles were adsorbed on to the coverslips by filling 10-ml test tubes with a suspension of 10^7–10^8 particles per ml. The coverslip was then introduced and the tube stoppered. Adsorption was allowed to continue for various times up to 20 h when the coverslips were removed and washed in a stream of distilled water.

In order to economize on the volumes required, this technique was slightly modified for the adsorption of the virus particles. An 0.1 ml volume of the labelled

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virus suspension was run between the coverslip and the inside of the test tube which had been treated with silicone.

Counts of adsorbed particles

The number of latex particles adsorbed on to each film was counted directly with the electron microscope. The mean number of particles per field at a magnification of about × 6000 was found and the area of the field measured in terms of the diameter of the latex particles.

The suspension of the largest latex particles (Batch LS-066-A) was adsorbed on to coverslips and counts made with a phase contrast microscope. The field was defined by a rectangular stop and its area measured with a micrometer eyepiece.

The fraction of the virus particles adsorbed on to the coverslips was measured by the ratio of the radioactive count which was found on the well washed coverslips after adsorption to that found when the whole of the added volume was dried down on to them.

The radioactive counts in the case of the ^{131}I isotope were measured with a sodium iodide crystal scintillation counter and the ^{32}P isotope with an end window Geiger counter.

RESULTS

When polystyrene latex or virus particles were adsorbed on to an aluminium surface, the number adsorbed in a given time was found to be independent of the concentration of electrolyte added to the suspension; in fact adsorption proceeded equally fast from distilled water. With all the other surfaces used, however, adsorption only occurred at the maximum rate when sufficient electrolyte was present and was slow or negligible from distilled water or sucrose solutions. Therefore, except in the case of aluminium surfaces, 1% sodium chloride was added to the suspensions. It was then found that the number of particles adsorbed per unit area in a given time was the same for every type of surface and in excellent agreement with the calculated number of particles colliding with the area as a result of Brownian motion (equations (2) and (9)). The actual numbers of latex particles (580-G) and vaccinia particles adsorbed from drops on to various films in 4 min and counted with the electron microscope are given in Table I.

According to theory (equation 9) it is the square of the number of adsorbed particles that should increase linearly with time and the fulfillment of this prediction is shown in Fig. 1 where the squares of the numbers of latex particles of two different sizes (580-G and LS-055-A) adsorbing on to carbon films and counted with the electron microscope have been plotted. The drawn lines were calculated from equations (2) and (9) and fit the observed points well.

To avoid any effects from evaporation, which was apt to occur despite the use of saturated atmospheres, these experiments with drops of suspensions adsorbing to films were only followed for times up to 30 min. Adsorption for longer times was studied by using coverslips immersed in suspensions of the largest latex particles (LS-066-A) and in this case the effect of varying degrees of shaking on the rate of adsorption was also investigated. In these longer experiments clumping of the latex suspensions was a complicating factor when salts were present and, to avoid this

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TABLE I

LATEX PARTICLES ADSORBED TO VARIOUS FILMS

A suspension in 1 % sodium chloride of latex particles of diameter 0.26 μ ($2.5 \cdot 10^{10}$ particles/ml and a suspension of vaccinia particles ($2.6 \cdot 10^{10}$ particles/ml) were adsorbed for 4 min at 21° ($\eta = .0.01$)

Adsorbing surface	*Particle count adsorbed* ($\pm$ SE*) $\cdot 10^{-7}$/cm²	
	Latex	*Vaccinia*
Nitrocellulose	5.3 ± 0.2	6.0 ± 0.1
Carbon	5.4 ± 0.1	6.1 ± 0.1
Aluminium	5.5 ± 0.2	6.2 ± 0.3
Gold	4.9 ± 0.3	5.6 ± 0.4
Brownian theory collision frequency**	5.6	6.3***

* Variation in the count per field shown to have a Poisson distribution.
** Calculated from equations (2) and (9).
*** Particles assumed to be spheres of radius 0.11 μ.

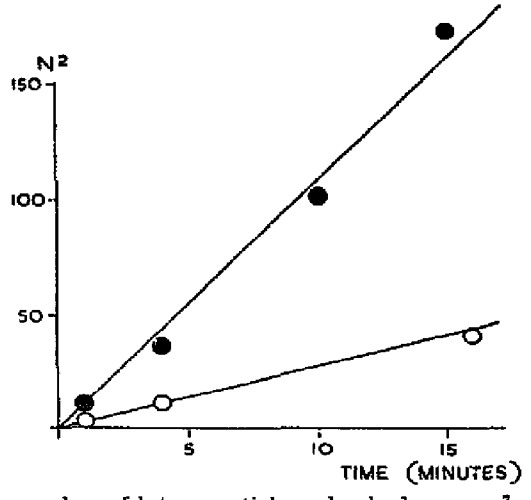

Fig. 1. The square of the number of latex particles adsorbed per 10^{-7} cm² as a function of time. ○ Latex 0.26 μ diameter in suspension containing $1.5 \cdot 10^{10}$ particles/ml. ● Latex 0.16 μ diameter in suspension $2.3 \cdot 10^{10}$ particles/ml. The drawn lines give the theoretical values of the numbers of particles colliding with the surface due to Brownian motion calculated from equations (2) and (9).

difficulty, the coverslips were aluminized so that distilled water suspensions could be used. Adsorption was allowed to proceed under three conditions of agitation —with the tubes standing in a rack (which will be referred to as "at rest"), with tubes continuously rotated in a rolling device at 1 revolution in 6 min ("rolled") and more violently shaken by a mechanism which inverted the tubes 3 times a min ("shaken"). Rather unexpectedly, the counts of adsorbed particles (made with the phase contrast microscope) were almost identical in each of these systems and even over periods of up to 18 h all still followed the theoretical number of Brownian collisions (Table II). The experiment was repeated with vaccinia and fowl plague virus particles suspended in sucrose and adsorbed "at rest" on to aluminized coverslips. The fractions of the virus adsorbed after times up to 20 h as given by the radioactive counts are shown in Table III. Again the theoretical values deduced from equation (7) were closely followed.

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TABLE II

ADSORPTION OVER LONG TIMES WITH VARYING AGITATION

A suspension of latex particles of diameter 0.09 μ ($1.5 \cdot 10^7$ particles/ml) adsorbed on to aluminium surfaces with varying degrees of agitation at 21° ($\eta = 0.01$).

Time (h)	Particle counts adsorbed (± SE*) · 10^{-3}/cm²			
	At rest	Rolled	Shaken	Brownian theory**
0.5	6.2 ± 0.5	6.5 ± 0.5	—	5.6
1	7.5 ± 0.6	—	—	8.0
2	10.4 ± 0.4	12.3 ± 0.5	—	11.2
4	17.9 ± 0.8	21.7 ± 0.8	—	15.9
18	40.4 ± 1.8	32.4 ± 1.8	20.5 ± 2.2	33.7

* Variations in the count per field shown to be Poissonian in all cases.
** Calculated from equations (2) and (9).

TABLE III

ADSORPTION OF VIRUS PARTICLES TO ALUMINIUM SURFACES

Particles suspended in 0.25 M sucrose ($\eta = 0.012$ at 21°) and adsorbed from a layer 0.1 cm thick.

	Time (h)	% particles adsorbed	
		Experiment	Brownian theory*
Vaccinia**	3	14	15
	20	35	39
Fowl plague***	0.25	7	7
	0.5	11	10
	1	14	14
	2	21	20
	20	56	63

* Calculated from equations (2) and (7).
** Assumed spherical with radius 0.11 μ.
*** Assumed spherical with radius 0.04 μ.

The effect of ions on adsorption

As already mentioned, the rate of adsorption of particles on to an aluminium surface was independent of the salt concentration, but for adsorption on to the other surfaces investigated (nitrocellulose, carbon, gold and glass) the maximum rate was only reached when an adequate concentration of ions was provided. In these cases a typical curve was obtained when the logarithm of the number of particles adsorbed on unit area in a given time was plotted against the logarithm of the concentration of salt in the suspension. Fig. 2 shows the results of an experiment in which latex particles (580-G) were adsorbed on to nitrocellulose films from suspensions containing various strengths of sodium chloride. Plotted as described, the points lie on a line which rises linearly until the adsorbed count equals the Brownian collision frequency and then continues at this level for all higher concentrations of salt. (A slight fall at very high salt concentrations was sometimes observed, but this was accounted for by clumping of the particles in suspension.) A similar plot was found with all the other surfaces and with the different particles. Each curve can thus be represented

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by two parameters, first the slope of the initial rising portion (*i.e.* δ (log N)/δ(log c) when N is the number of adsorbed particles in a given area and c is the concentration of salt: this slope is independent of the units in which N and c are expressed) and second the minimum salt concentration at which the maximum adsorption rate is reached. This latter point was about 0.1% sodium chloride for latex adsorbing on to nitrocellulose and was similar for the glass and carbon surfaces. The figures obtained, however, were not very reproducible and varied not only with the type of surfaces involved but also from batch to batch of similar surfaces. The highest requirement found was 5% sodium chloride for vaccinia particles adsorbing on to a gold surface.

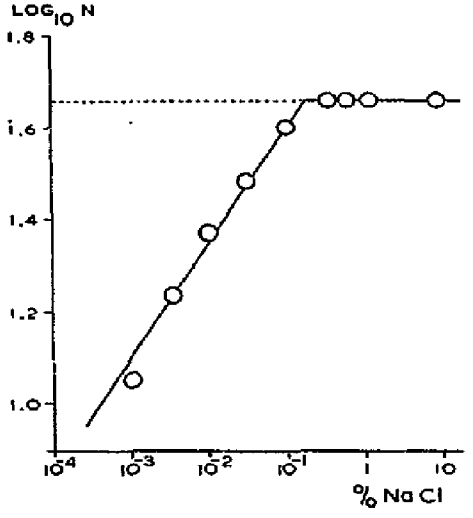

Fig. 2. The logarithm of the number of latex particles (0.26 μ diameter; $2.55 \cdot 10^{10}$ particles/ml) adsorbed per field ($1.6 \cdot 10^{-6}$ cm^2) in 1 min as a function of the logarithm of the salt concentration. The dashed line shows the theoretical Brownian collision frequency. The slope of the initial rising portion of the graph (δ log N/δ log c) is 0.25.

On the other hand, the slope of the initial portion of the curve showed a remarkable constancy and reproducibility. Within the limits of experimental error, it was independent of the nature of the adsorbing surface. It varied with the different sizes of latex but with each of the two types of virus particle was the same as for the correspondingly sized latex. There was little difference between the results with sodium chloride and those obtained with other univalent cations, but the slope was doubled when divalent cations (Ca^{++} and Mg^{++}) were used. Trivalent aluminium salts gave a slope that was not significantly higher than the divalent salts. The valency of the anion had no effect on the results. The results with the various particles, surfaces and salts are summarized in Fig. 3 which shows the value of initial constant slope δ (log N)/δ(log c) plotted against the size of the particle being adsorbed in the presence of various salts.

Effect of soluble protein

Some experiments with inadequately purified suspensions of virus particles adsorbing on to the films on electron microscope specimen supports gave unexpectedly low rates of adsorption; consistent results were obtained only after further purifica-

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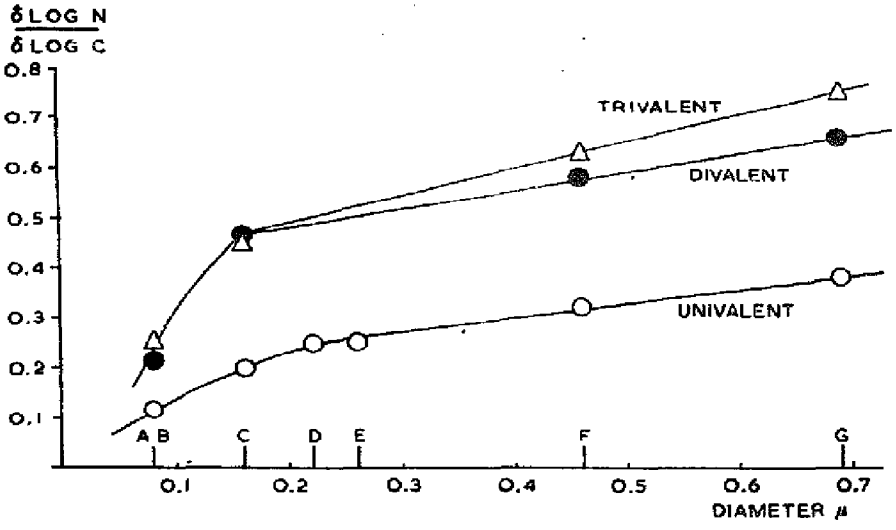

Fig. 3. The slope (δ log N/δ log c) of the initial rising portion of plots of the type shown in Fig. 2 as a function of the diameter of the particle being adsorbed from suspensions in various salts: ○ Univalent (NaCl or KCl); ● divalent ($MgCl_2$ or $CaCl_2$); △ trivalent ($AlCl_3$). A, latex 0.08 μ diameter; B, fowl plague virus; C, latex 0.16 μ diameter; D, vaccinia virus; E, latex 0.26 μ diameter; F, latex 0.46 μ diameter; G, latex 0.69 μ diameter.

tion. This was supposed to be due to the presence of soluble protein which, by adsorbing on the surface involved, conferred hydrophilic properties on it and thus hindered the adsorption of the particles. This conclusion was confirmed by adding 1% bovine albumin to a suspension of latex particles. The count of adsorbed particles fell to 10% of the maximum Brownian rate and was independent of the salt concentration over the range 0.01% to 18% sodium chloride. In the absence of the albumin, 0.1% or more sodium chloride gave the maximum adsorption rate. Similar results were obtained when vaccinia and fowl plague viruses were adsorbed on to glass, the count falling in the presence of 5% serum to 7.2% and 9.8% respectively of the count obtained when the particles were adsorbed from GEY's solution without serum. When the glass coverslips were treated with silicone an even more marked depression was observed.

DISCUSSION

At first sight, perhaps the most unexpected feature of this investigation has been the finding that the rate at which particles of the size of viruses come into collision with a surface can be almost exactly predicted from Brownian theory even when the suspension is being well and continuously shaken. This is almost certainly to be explained by supposing that in contact with the surface is a layer of fluid 0.1–1.0 mm thick which remains virtually at rest although the rest of the fluid may be in motion. An assumption of this sort is in fact the well-established basis of the theory of viscous flow. Now even large molecules can diffuse rapidly through such a layer and the rate at which they react with a surface can consequently be considerably increased by shaking the system. On the other hand, particles the size of viruses

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(say between 500 and 2500 Å diameter) in Brownian motion will take on an average about 4 h to diffuse 0.1 mm and some 15 days to diffuse 1 mm. The presence of a layer of stationary fluid above a surface thus presents a very considerable barrier and even a shaken suspension is still virtually diffusion-limited.

This finding, however, makes it easy to give a reliable estimate of the fraction of the virus particles in a suspension that can reach a surface in a given time, valid at least for times up to 20 h. The diffusion constant of the virus, if the particles can be assumed to be reasonably spherical, is first calculated from equation (2); the result will be sufficiently accurate even if only a rough estimate of particle size can be given. The value obtained is then substituted either in equation (7) or (8), whichever is appropriate, if the surface is approximately flat, or in equation (16) for adsorption on to cells in suspension. The fraction of particles adsorbed after various times is then given.

Such calculations at once suggest that there should be a very variable delay in the start of infection after the virus is added to a cell system. For even if only a thin layer of virus suspension lies above the cells it will still be at least a matter of hours before half the particles can have reached a cell surface. Such a phenomenon has in fact already been observed experimentally. CAIRNS[19] has reported that when influenza virus infects the allantoic cells of eggs there is a delay of about 8 h before half the virus has initiated an infection.

In general terms, the observations on the effect of ions on adsorption are readily explained. Both the latex and the virus particles carry a nett negative charge at pH values close to neutrality. An aluminium surface is positively charged and in this case, therefore, there is no barrier to adsorption. Hence adsorption always occurred at the calculated rate at which the particles diffused to the aluminised surface. All the other surfaces investigated were negatively charged and there was in consequence an electrostatic barrier to adsorption of just the same kind as that responsible for the stability of a suspension of hydrophobic particles, *i.e.* the layer of similarly charged ions that extends from the surface of each particle out into the fluid. The thickness of this layer can be reduced by raising the ionic strength of the suspending fluid. Above a certain strength, the surfaces of the particles can then come sufficiently close for short range attractive forces to bind them together. That such a process in outline explains the need for the presence of dissolved salts before either flocculation or adsorption can occur would be generally agreed. Probably the most satisfactory, and certainly the most elegant, account along these lines attempting a quantitative treatment of the problem is that of VERWEY AND OVERBEEK[20,21]. Their treatment deals only with the interaction of similar surfaces, but it has been extended by others to give an interesting account of the adsorption of phage to bacteria[22,23], apparently with some measure of success. There are, however, theoretical objections to the extension of the theory to interaction between dissimilar surfaces[24] so that, despite the obvious need for such a general theory in many branches of biology, any application of this sort must be carefully examined. Certainly our results seem to be in disagreement with the quantitative predictions of the extended theory, since it is possible to use the theory to obtain an expression[25] for the fraction M of the collisions between surfaces that result in firm attachment as a function of the salt concentration c. Now this fraction M is not strictly proportional to the number of adsorbed particles because some of these may have made several unsuccessful

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collisions before finally becoming attached, but when the rate of adsorption is nearly equal to the Brownian collision frequency, our slope $\partial \log N/\partial \log c$ should be nearly the same as $\partial \log M/\partial \log c$ (if anything it will be larger). According to the theory $\log M$ should be proportional to $\log c$ as, indeed, we have repeatedly found $\log N$ to be. But the resulting constant slope $\partial \log M/\partial \log c$ should vary in direct proportion to the particle size and decrease when a salt of a higher valency is used. In our experiments, the slope was not proportional to particle size and increased when a salt of higher valency was used. Again, the predicted slopes for univalent salts are of the order of 10 for the size of particle we used, while our observed slopes have values of around 0.25. Thus we find the theory of VERWEY AND OVERBEEK does not give a satisfactory account of our results on interactions between dissimilar surfaces.

The observation that divalent ions double the value of the slope $\partial \log N/\partial \log c$ found with univalent ions but that trivalent ions have only a slight additional effect suggests that perhaps the slope is a function of the size of the ions rather than their valency. This would parallel our finding that it is the particle size and not apparently its surface charge that is related to the slope.

The method of counting the number of particles adsorbed to the films on electron microscope specimen supports provides a very simple technique for estimating the particle count in a suspension. As already mentioned, however, the method gives a reliable figure only if the suspension is free from soluble protein.

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