VIRUS PARTICLE ADSORPTION

III. ADSORPTION OF VIRUSES BY CELL MONOLAYERS AND EFFECTS OF SOME VARIABLES ON ADSORPTION

A. C. ALLISON AND R. C. VALENTINE

National Institute for Medical Research, Mill Hill, London (England)

(Received September 7th, 1959)

SUMMARY

The rates of adsorption of ^{32}P-labelled fowl plague virus and ^{131}I-labelled vaccinia virus by monolayers of chick embryo and HeLa cells have been investigated. With both types of virus and cell the observed rates of adsorption were very nearly one half the rates expected from Brownian theory and observed when virus was adsorbed by glass and other non-biological surfaces. The adsorption of viruses by cells is not depressed by protein. Adsorption of viruses by cells is dependent on the concentration of cations in the medium, and is depressed by high concentrations of multivalent cations. The rate of adsorption is unaffected by heat inactivation, weak formalin treatment and thiol reagents. The rate of adsorption is increased by low concentrations of polycations and in the presence of high concentrations of uranyl ions; it is diminished by low concentrations of polyanions and by acetylation of the amino groups of the virus. It is suggested that the main interacting groups are the amino groups of the virus and phosphate groups of the host cell wall. The effects of ions on adsorption cannot be explained by current theories describing the interaction of charged surfaces.

INTRODUCTION

In the first paper of this series[1] equations were developed from the theory of Brownian motion defining the rate of arrival of particles in suspension at a surface. The actual rates at which virus and other particles of similar size are adsorbed by nitrocellulose, glass, carbon, gold and aluminium surfaces were investigated. It was shown that, even when the suspensions were agitated, under optimal conditions the proportions of particles adsorbed agreed closely with theoretical expectation.

In the second paper of the series[2] the kinetics of adsorption of viruses by suspensions of red blood cells, ascites tumour cells and tissue culture cells were analysed. The rates of adsorption were found to be appreciably lower than theoretical expectation from the calculated frequency of collisions between virus particles and cells. The rates of adsorption of radioactively labelled vaccinia and fowl plague viruses by cell monolayers are described in the present paper. Again, the observed adsorption is significantly less than that expected from Brownian theory, although the deficiency

is not as marked as with cells in suspension. In an attempt to explain the less efficient adsorption of viruses by cells than by non-biological surfaces and to define some of the factors influencing adsorption of animal viruses to cells, the conditions of adsorption, charge of adsorbing surfaces (ionic strength, hydrogen ion concentration, and temperature) have been varied, and the results of these experiments are also outlined and discussed.

MATERIALS AND METHODS

Radioactively labelled virus

The preparation of purified vaccinia virus labelled with ^{131}I and fowl plague virus labelled with ^{32}P, and the techniques of measuring radioactivity, were previously described[1].

Cell monolayers

Monolayers of chick embryo tissues in 5-cm and 6-cm diameter Petri dishes were prepared by the method of DULBECCO[3]. In some experiments HeLa cell monolayers were prepared by seeding the Petri dishes with $5 \cdot 10^7$ cells in 5 ml growth medium (70 parts Gey's solution; 10 parts human serum; 10 parts of 1 % yeast extract in Gey's solution; and 10 parts 5 % lactalbumin hydrolysate in Gey's solution), and the cells incubated until they formed a uniform sheet on the bottom of the Petri dish.

Adsorption was measured by adding 0.5 to 2 ml of labelled virus in the appropriate medium to the washed cell monolayer, leaving for various times of adsorption and terminating the process by rapidly removing the supernatant fluid and washing the cell monolayer repeatedly with the same medium but no virus. Appropriate controls showed that there was no significant elution of virus from the cells during the course of washing or preparation for counting.

The cells were suspended by incubation with 0.1 % trypsin in Gey's solution and the radioactivity of the cell suspension measured. For ^{131}I counts, the cell suspension was made up to 2 ml, placed in standard tubes and counted in a well-type scintillation counter. For ^{32}P counts the cell suspension (evenly spread with a small quantity of detergent) was dried down onto 1-cm^2 planchettes and assayed in a Geiger end-window counter.

THEORETICAL

It has been deduced[1] that the number, N, of particles suspended in fluid reaching a unit area of flat surface in contact with the fluid is given, for less than 40 % of the particles adsorbed, by

$$N = 11.3\, c_0 \sqrt{Dt} \qquad (1)$$

where c_0 is the initial concentration of particles in the fluid (number/unit volume), D is the diffusion coefficient of the virus and t is the time.

For more than 28 % of the particles adsorbed,

$$N = c_0 d \left(1 - \frac{8}{\pi} \exp\{-\pi^2 Dt/4d^2\}\right) \qquad (2)$$

where d is the depth of fluid above the surface.

The diffusion coefficient of the virus, D, can be calculated from the STOKES-EINSTEIN equation:

$$D = \frac{kT}{6\pi\eta a} \tag{3}$$

where k is BOLTZMANN's constant ($1.38 \cdot 10^{-16}$), T is the absolute temperature, η the viscosity of the suspending fluid and a the radius of the particles.

Previous experiments[1] showed that, when virus and polystyrene latex particles suspended in fluid in the neighbourhood of neutrality are adsorbed by surfaces of nitrocellulose, glass, carbon and gold, the observed rate of adsorption is close to expectation from Brownian theory (equations 1 and 2), provided the concentration of cations is sufficient. The same is true of adsorption by positively charged aluminium surfaces even in the absence of ions.

This result follows from the net negative charge of the virus above pH 5.6 which, allows adsorption by positively charged surfaces under a wide range of conditions. Adsorption by negatively charged surfaces, on the other hand, can only take place when the concentration of cations is sufficient to reduce the thickness of the ionic double layer on the two surfaces, which can then come close enough together for short-range attractive forces to bring about adsorption.

The presence of even small amounts of protein strongly depresses attachment of virus particles to non-biological surfaces. It was suggested[1] that the rapidly diffusing protein soon becomes adsorbed on to the surface, conferring on it hydrophilic properties and so hindering the attachment of virus or polystyrene latex particles.

EXPERIMENTAL

Rate of attachment of virus to cell monolayers

Experiments were carried out with ^{32}P-labelled fowl-plague virus and ^{131}I-labelled vaccinia virus in Gey's solution without bicarbonate. The rates of attachment to chick embryo cell monolayers are shown in Figs. 1 and 2. Very similar results were obtained with HeLa cell monolayers.

As expected from Brownian theory, a straight line is obtained when adsorbed virus is plotted against the square root of time. For both viruses the rates of adsorption by cell monolayers are very nearly one half of the maximum theoretical rate. The addition of calf serum in concentrations up to 5 % by volume made no significant difference to this result.

At 4° the rate of adsorption was reduced to 64 % of that at 20°. This is in agreement with the factor of 57 % expected from the increased viscosity of the medium at 4°, and implies that the adsorption of virus by cells is not dependent on temperature.

The adsorption of infective virus was followed by using unlabelled virus in higher dilution and recording as a function of adsorption time the number of plaques produced by fowl plague virus in chick embryo cell monolayers with the standard bicarbonate-agar overlay[3] and vaccinia virus with tris(hydroxymethyl)aminomethane-buffered agar overlay[4]. Representative results are given in Fig. 3 and Table I. Again the number of plaques increases in proportion to the square root of the adsorption time.

The validity of equation (2) was also tested by varying the volume of the fluid in the inoculum, which affects the term d (depth of fluid containing virus above

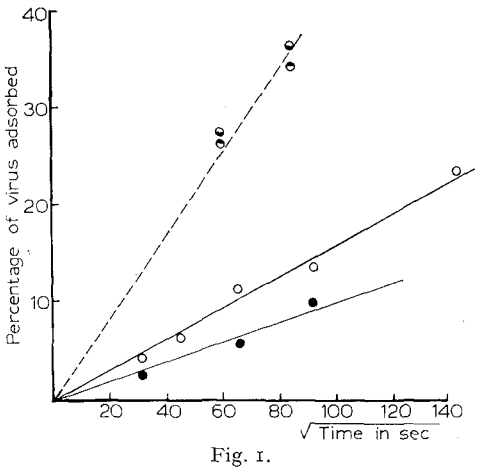

Fig. 1.

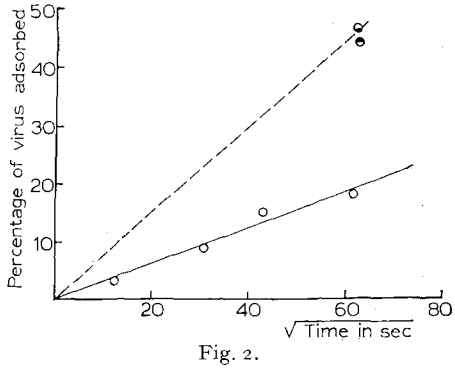

Fig. 2.

Fig. 1. Adsorption of labelled vaccinia virus by cell monolayers in 6-cm diameter Petri dishes. Interrupted line: percentage of virus colliding with monolayer at 20° calculated from Brownian theory. The percentages of virus adsorbed by glass (◓) and aluminium (◒) surfaces, and by chick embryo cell monolayers (○) at 20° and (●) at 4° are shown.

Fig. 2. Adsorption of labelled fowl plague virus by chick embryo cell monolayers ○ glass ◓ and aluminium ◒ surfaces, compared with the maximum calculated from Brownian theory (interrupted line).

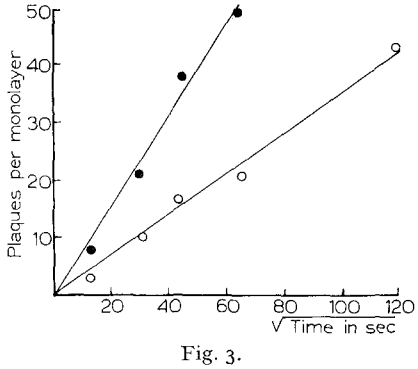

Fig. 3.

Fig. 3. Increase in the number of vaccinia ○ and fowl plague ● plaques in chick embryo cell monolayers as a function of the square root of the time of adsorption.

TABLE I

EFFECTS OF INOCULUM VOLUME AND ADSORPTION TIME ON FOWL PLAGUE VIRUS PLAQUE PRODUCTION IN CHICK EMBRYO CELL MONOLAYERS (MEAN OF 4 DISHES)

Inoculum volume	*Time of adsorption (min)*	*Mean plaque count*
0.5 ml	15	13.5
	30	17.2
	60	29.0
	120	31.8
1.0 ml	15	18.2
	30	26.0
	60	37.5
	120	41.0
2.0 ml	15	19.8
	30	27.0
	60	36.5
	120	43.2

monolayer). Typical results are given in Table I. It is clear that, in accordance with the predictions of the equation, increasing the volume of fluid from 0.5 ml to 1 ml does not double the number of plaques produced, and increasing the volume to 2.0 ml results in only a small further increase in number of plaques.

In plaque titrations it is convenient to use a fairly large volume of fluid containing appropriate dilutions of virus (at least 0.5 ml) so as to have an even layer over the cells. In assaying infectious units it can be taken that the fraction of virus adsorbed by the cell monolayer is, to a close approximation, one half of that calculated from equation (2)[4].

Effect of ions on adsorption

Chick embryo cell monolayers were washed in 0.25 *M* sucrose and virus dilutions in sucrose, together with the appropriate concentrations of various salts, were added for 1 h. In calculating the maximum rate of adsorption from Brownian theory, a correction was introduced for the change in viscosity of the medium produced by sucrose.

The main results are summarized in Fig. 4. Adsorption takes place, not only with divalent or trivalent ions, but also in the presence of univalent ions alone, provided their concentration is sufficiently high. This was confirmed by carrying out experiments in the presence of 0.01 *M* sodium ethylenediaminetetraacetate, which chelates the multivalent ions in the media used. The rate adsorption was then reduced to that expected in the presence of the corresponding univalent ions alone.

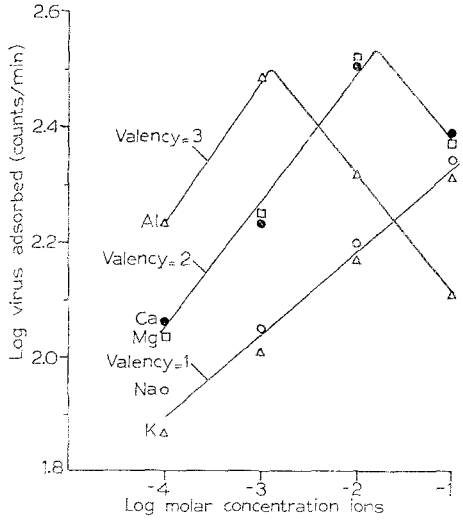

Fig. 4. Effect of cations on adsorption of labelled fowl plague virus by chick embryo cell monolayers. Count expected from Brownian Theory is 2·82.

As with adsorption by non-biological surfaces[1], straight lines are obtained when the logarithm of the adsorbed virus is plotted against the logarithm of the concentration of ions, the slope being much steeper for divalent ions than for univalent, and slightly steeper again for trivalent ions. At high concentrations, however, in contrast to the findings with non-biological surfaces, divalent and trivalent ions

depress adsorption to cell monolayers. The symmetrically rising and falling curves shown in Fig. 4 are obtained. Adsorption was independent of the valency of the anions present, and control experiments showed again that there was no significant elution of virus from cells under the conditions used.

Effects of polyelectrolytes on adsorption

To obtain further information about the electrostatic interaction of viruses and cells, adsorption was carried out in the presence of various polyelectrolytes: chondroitin sulphate and a substituted benzene sulphonic acid polymer (53K)[5], protamine and polylysine. In some experiments adsorption was carried out in the presence of polyelectrolytes, in others the polyelectrolytes were added to the cells or virus and the excess removed. The cell monolayers and virus were then washed once with appropriate medium lacking the polyelectrolyte (virus by high speed centrifugation followed by ultrasonic dispersion). It could be shown that both cells and virus take up ^{14}C-labelled 53K in the presence of Ca^{++} and Al^{+++} ions used in representative experiments.

The main results are summarized in Table II. It is evident that both polyanions, whether present in the adsorbing medium or added to cells or virus beforehand, suppress adsorption, whereas both polycations in low concentrations enhance it. In higher concentrations protamine added to the virus, but not to the cells, depressed adsorption, presumably because it brought about clumping of the virus.

TABLE II

EFFECT OF POLYELECTROLYTES ON ADSORPTION OF ^{32}P-LABELLED FOWL PLAGUE VIRUS BY CHICK EMBRYO CELLS

Control in Gey's salt solution without bicarbonate. Polyelectrolyte concentration 1 mg/ml Gey's solution unless stated. Adsorption is expressed as a percentage of the maximum calculated from Brownian theory (equation 2).

Polyelectrolyte	*Where added*	*Adsorption*
Control	—	48
Protamine	Fluid	76
Protamine	Cells	82
Protamine	Virus	68
Protamine 10 mg/ml	Cells	72
Protamine 10 mg/ml	Virus	28
Polylysine	Fluid	68
Polylysine	Cells	76
Polylysine	Virus	70
Hyaluronic acid	Fluid	27
Hyaluronic acid	Cells	31
Hyaluronic acid	Virus	22
53K (substituted benzene sulphonic acid polymer)	Fluid	22
	Cells	23
	Virus	14

In Fig. 5 the effects of trivalent ions on adsorption in the presence of polyanions and polycations are shown. In the control, as before, low concentrations of Al^{+++} ions

promote adsorption, whereas high concentrations depress it. In the presence of polylysine, intermediate concentrations of Al^{+++} ions increased adsorption virtually to the maximum rate calculated from Brownian theory, but high concentrations again depressed adsorption. In the presence of 53 K adsorption was low throughout, but was not depressed by high concentrations of Al^{+++} ions.

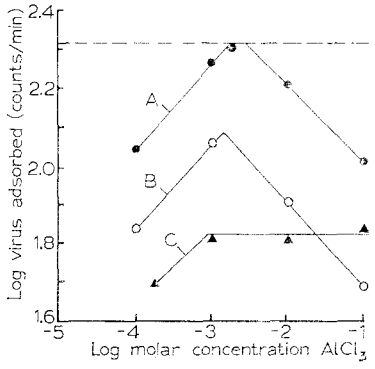

Fig. 5. Effect of concentration of Al^{+++} ions on adsorption of labelled fowl plague virus on chick embryo cells untreated (B), treated with polylysine (A) and with substituted benzene sulphonic acid polymer (C). The interrupted line shows the count expected from Brownian theory.

Effects of other reagents on adsorption

The effects of some other reagents on adsorption were investigated (Table III). Agents reacting with sulphydryl groups of cells and virus, including Hg^{++}, *p*-chloromercuribenzoate, iodoacetamide, N-ethylmaleimide and ω-bromacetophenone, produced no detectable change in the rate of attachment of virus to cells, although all these agents markedly depress virus infectivity[6].

TABLE III

EFFECTS OF VARIOUS REAGENTS ON ADSORPTION OF ^{32}P-LABELLED FOWL PLAGUE VIRUS BY CELL MONOLAYERS

Reagent	*Adsorption*
Control	54
p-chloromercuribenzoate	51
Hg^{++}	58
N-ethylmaleimide	49
Iodoacetamide	51
ω-bromocetophenone	53
Heat	50
Formalin 0.1 %	48
Acetic anhydride	14
$(UO_2)^{++}$	76
Trimethylenediamine	74

Heating the virus at 68° for 0.5 h, which abolishes infectivity, does not affect the rate of attachment to cells. Low concentrations of formalin (0.1 % or less for 4 h at 37°) had no detectable effect on adsorption, but higher concentrations of formalin (1 %) for 4 h depressed adsorption. Treatment of the virus with 10^{-3} *M* acetic anhydride at pH 7.8 in bicarbonate buffer, followed by neutralization to pH 7.0, reduced the rate of adsorption quite considerably.

On the other hand, in the presence of uranyl ions in high concentration, adsorption

was increased. The same was achieved by trimethylenediamine in high concentration. The depressed adsorption shown with divalent cations was not apparent with this compound, in which the two positively charged groups are separated by three carbon atoms.

Effect of pH on adsorption

Fowl plague virus labelled with ^{32}P was made up in various buffers covering the range pH 3.0 to 7.0. Experiments above pH 7.5 failed because the cell monolayer lifted from the glass. The adsorption of virus as a percentage of the maximum calculated from Brownian theory is shown in Fig. 6. It will be seen that adsorption is greatest at pH 5.5, irrespective of the nature of the buffer ions present. Between pH 5.5 and 7.0 there is a slight decline in the efficiency of adsorption, and between pH 5.5 and 4.0 there is a marked decline. Below a minimum at pH 4.0 the percentage of virus adsorbed rose again.

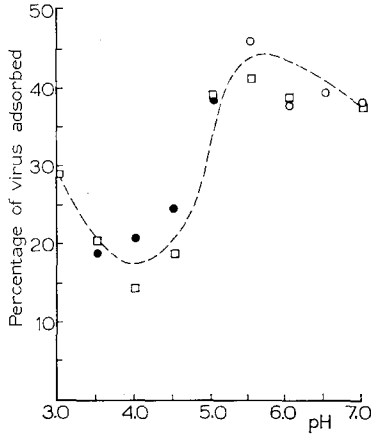

Fig. 6. Effect of pH on adsorption of fowl plague virus by chick embryo cell monolayers. Virus adsorbed is expressed as a percentage of the maximum value calculated from Brownian theory. Glycine acetate phosphate buffer ○, acetate buffer ●, phosphate buffer □.

DISCUSSION

Attempts have been made to use a rate constant of the type applicable to adsorption of phage by bacteria to describe the adsorption of animal viruses by cell monolayers:

$$f = 1 - \exp\{-Knt\}$$

where f is the fraction of virus adsorbed, n is the number of adsorbing cells, t is the time after commencing adsorption and K is the rate constant.

As previously pointed out[1], the use of such equations to define the rate of adsorption of virus by cell monolayers is not justified. Some measurements of adsorption of animal viruses by cell layers have already been published[7,8], but these have been based on infectivity determinations showing rather a wide scatter. Moreover, the conditions under which adsorption takes place have not been varied sufficiently to give useful information about the adsorption process.

In the case of phage[9–11] adsorption occurs efficiently in the presence of sufficient concentrations of cations. PUCK AND TOLMACH[12] concluded that the main interacting groups are the amino groups of phage and the carboxyl groups of host bacteria. DIRKX *et al.*[11] have had some measure of success in defining the conditions under

which phage adsorption takes place in terms of the ionic double layer theory of VERWEY AND OVERBEEK[13].

From our experiments certain general conclusions can be drawn. First is a point of practical use in experiments involving plaque titrations and quantitative studies of virus growth. The rate of attachment of virus to cell monolayers is very close to half the collision frequency calculated from Brownian theory. Thus by halving the value for N obtained by using equation (1), a good estimate of adsorbed virus can be made. This appears to hold, not only for the viruses studied by us (vaccinia and fowl plague), but also for herpes simplex[7] and poliomyelitis[8], four viruses that probably differ appreciably in surface composition. Adsorption to non-biological surfaces, on the other hand, proceeds at a rate almost identical with the collision frequency[1]. Another difference is apparent when protein is added to the medium. No effect on adsorption of viruses by cells was detected, but adsorption by glass and other surfaces was strongly depressed. This again has practical application, for it provides a way of reducing loss of virus through adsorption to glassware without interfering with adsorption to cells.

As already found for phage, adsorption of animal viruses to host cells requires adequate concentrations of cations. However, the usual implication that these must be divalent is incorrect, since adsorption takes place in the presence of adequate concentrations of univalent cations alone. Thus the concept that adsorption results from the formation of divalent cationic bridges between viruses and cells is invalidated. Moreover, the results are not in agreement with the quantitative predictions of the double ionic layer theory of VERWEY AND OVERBEEK[13]. As in the adsorption of viruses to inorganic surfaces, increasing the valency of the cation increases the slope of the log adsorbed virus against log concentration of ion; whereas according to their theory the slope should decrease with cations of higher valency. In addition the theory of VERWEY AND OVERBEEK does not explain the suppression of adsorption by high concentrations of divalent and trivalent cations.

Third, some tentative conclusions can be drawn about the interacting surfaces. Although influenza virus (which is related to fowl plague) has about 11.5 % phospholipid[14], electrophoretic measurements show that it is isoelectric at about pH 5.3[15]. In other words the surface charge is dominated by amino and carboxyl groups of protein rather than strongly acidic groups of phospholipid. The animal cells that have been studied retain a negative charge at a much lower pH, and the electrophoretic mobility in the presence of uranyl and other ions suggests that the surface charge is dominated by strongly acidic, especially phosphate, groups[16]. At pH 5.5 to 7.6—the conditions under which most experiments are undertaken—cells and virus particles are both negatively charged. There is thus an electrostatic barrier to adsorption in the form of layers of positively charged ions concentrated around the surfaces of both cells and viruses. The thickness of this layer can be reduced by raising the concentration of cations in the suspending fluid. Above a critical concentration the virus can come sufficiently close to the cells for short range attractive forces to bind them together. The temperature coefficient, effect of ions and other properties make it clear that the primary attachment is electrostatic. The main interacting groups are likely to be amino groups of the virus and strongly acidic, mainly phosphate, groups of host cells. This is shown by mild acetylation of the amino groups of the virus, which depresses adsorption to host cells (Table III), although it makes no

detectable difference to the attachment of virus particles to positively charged aluminium surfaces. Acetylation of proteins under these conditions does not denature them, but lowers the isoelectric point considerably[17]. The effect of uranyl ions in promoting adsorption is consistent with the well-known affinity of these ions for phosphoric acid groups[16,18]. Agents reacting with sulphydryl groups of cells and viruses were without effect.

The increase in adsorption of virus between pH 7.0 and 5.5 can plausibly be explained by suppression of ionization of carboxyl-groups, reducing the net negative charge of the virus and enhancing adsorption. At about the isoelectric point of the virus there is considerable aggregation of virus particles to form large, slowly diffusing units, so that the rate of attachment to cells is much reduced.

As expected, polyanions which increase the net negative charge of both host cells and virus, or each separately, diminish adsorption. Low concentrations of polycations, on the other hand, increased adsorption. Higher concentrations added to cells increased adsorption, but added to virus particles they decreased it, probably owing to aggregation of virus particles.

The reduction in virus infectivity in the presence of benzene sulphonic acid reported by HEYMANN *et al.*[19] may be due simply to suppression of adsorption. The interaction with ribonuclease quoted by these authors is only one example of the reactivity of polyanions of this type towards the basic groups of a wide range of proteins[20]. Reduction of virus infectivity in the presence of high concentrations of lysozyme may be analogous to the suppressed adsorption noted in our experiments in the presence of high concentrations of protamine, and is probably due to aggregation of virus particles.

The most puzzling observation is the suppression of adsorption of virus to cells (but not to glass and other surfaces) by high concentrations of divalent and trivalent cations. The relevant difference between biological and non-biological surfaces is likely to be that the former possess both positively and negatively charged groups whereas the latter have only negative charges. An important feature is illustrated in Figs. 4 and 5. When the logarithm of the adsorbed virus is plotted against the logarithm of the concentration of cations, linear increases are obtained. The slopes depend upon the valency of the cations and the size of the particles adsorbed, and have the same values for adsorption on to cells and non-biological surfaces. At certain ionic concentrations, however, in the case of adsorption to cells, the rising curve abruptly changes to a falling one, the slope remaining unaltered. It is this sudden change of sign that prevents the curve from rising to give an adsorption rate equal to the collision frequency, as is found with non-biological surfaces. The inhibited adsorption in the presence of multivalent cations is not due to elution, which was excluded in some experiments. The symmetry of the rising and falling curves does not suggest the intervention of an entirely new factor.

Existing theoretical analyses of the interactions between dissimilar charged surfaces during adsorption are far from complete[21]. It is possible that firm cation binding by both surfaces, of the type analysed by STERN[22], plays a part in the depressed adsorption of viruses in high concentrations of multivalent ions. The suggestion[1] that it is the size of ions as much as their charge that determines their effectiveness in promoting adsorption is consistent with this interpretation.

ACKNOWLEDGEMENTS

We are indebted to Dr. H. G. PEREIRA for help in the preparation of radioactive fowl plague virus and some other experiments, to Drs. C. KAPLAN and A. S. MCFARLANE for supplying and iodinating the vaccinia virus, to Dr. H. J. ROGERS for donating polylysine and substituted benzene sulphonic acid polymer (including a ^{14}C-labelled sample) and to Mrs. P. SMITH for technical assistance.

REFERENCES

1 R. C. VALENTINE AND A. C. ALLISON, *Biochim. Biophys. Acta*, 34 (1959) 11.
2 A. C. ALLISON AND R. VALENTINE, *Biochim. Biophys. Acta*, 40 (1960) 393.
3 R. DULBECCO, *Proc. Natl. Acad. Sci. Wash.*, 38 (1957) 747.
4 J. S. PORTERFIELD AND A. C. ALLISON, *Virology*, 10 (1960) 233.
5 H. J. ROGERS AND P. C. SPENSLEY, *Biochim. Biophys. Acta*, 13 (1954) 293.
6 A. C. ALLISON, unpublished observations.
7 J. S. YOUNGNER, *J. Immunol.*, 76 (1956) 288.
8 A. S. KAPLAN, *Virology*, 4 (1957) 435.
9 T. T. PUCK, *Cold Spring Harbor Symposia Quant. Biol.*, 18 (1953) 149.
10 L. J. TOLMACH, *Advances in Virus Research*, 4 (1957) 63.
11 J. DIRKX, J. BEUMER AND M. P. BEUMER-JOCHMANS, *Ann. inst. Pasteur*, 93 (1957) 340.
12 T. T. PUCK AND L. J. TOLMACH, *Arch. Biochem. Biophys.*, 51 (1954) 229.
13 E. J. W. VERWEY AND J. TH. G. OVERBEEK, *Theory of the Stability of Lyophobic Colloids*, Elsevier, Amsterdam, 1948.
14 L. H. FROMHAGEN, N. K. FREEMAN AND T. C. A. KNIGHT, *Virology*, 5 (1958) 173.
15 G. L. MILLER, M. A. LAUFFER AND W. M. STANLEY, *J. Exptl. Med.*, 80 (1944) 549.
16 A. D. BANGHAM, B. A. PETHICA AND G. V. F. SEAMAN, *Biochem. J.*, 69 (1958) 2.
17 R. M. HERRIOTT, *Advances in Protein Chem.*, 3 (1947) 170.
18 A. ROTHSTEIN AND R. MEIER, *J. Cellular Comp. Physiol.*, 38 (1951) 245.
19 H. HEYMANN, Z. R. GULICK, C. J. DE BOER AND G. DE STEVENS, *Arch. Biochem. Biophys.*, 73 (1958) 366.
20 H. NOGUCHI, *Biochim. Biophys. Acta*, 22 (1956) 459.
21 A. BIERMAN, *Proc. Natl. Acad. Sci. Wash.*, 41 (1955) 245.
22 O. STERN, *Z. Elektrochem.*, 30 (1924) 508.

LIGHT-SCATTERING STUDIES ON ASCITES TUMOR CELL RNA

M. J. KRONMAN, S. N. TIMASHEFF, J. S. COLTER AND R. A. BROWN

Eastern Regional Research Laboratory, Philadelphia, Pa. (U.S.A.), Wistar Institute for Anatomy and Biology, Philadelphia, Pa. (U.S.A.)*

(Received October 3rd, 1959)

SUMMARY

The molecular parameters of the high molecular weight, ribonucleic acid, prepared from Ehrlich ascites tumor cells have been determined. This RNA appears to consist of two main components of molecular weights of $2.3 \cdot 10^6$ and $3.2 \cdot 10^5$. Under conditions close to physiological, these molecules can be described best as compact rods 40–45 Å in thickness.

* Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.