higher in nephrotic animals, the difference was insignificant in the presence of the marked rise in chylomicronemia produced by injection of the test load of chyle. Furthermore, the presence of massive endogenous hyperlipemia in non-nephrotic rats did not alter rate of removal of administered chylomicrons. It is therefore concluded that the decreased rate of chyle removal from nephrotic plasma was not due to isotope dilution.

An inhibitor of lipoprotein lipase has been demonstrated in the serum of nephrotic rats (3), and the possibility was thus raised that the decreased rate of removal of chylomicrons is a consequence of this inhibition. Our results confirm the observation that nephrotic plasma inhibits the clearing action *in vitro* of post-heparin plasma. However, when normal rats were injected with nephrotic serum to obtain a concentration in their vascular system comparable to that used in the *in vitro* system, no inhibitory effect on rate of chyle removal was observed. This suggests that the clearing process may not play a major role in chylomicron removal. There is evidence that chylomicrons can be removed as particulate matter without prior hydrolysis by serum lipoprotein lipase(8), so that even if the degree of inhibition of lipoprotein lipase observed *in vitro* had obtained *in vivo,* it might not have been apparent in overall estimates of the chylomicron removal rate.

Summary. 1. Carbohydrate feeding reduced chylomicronemia to similarly low levels in normal and nephrotic rats; following such dietary preparation the decreased rate of chylomicron removal from blood of nephrotic rats is not due to dilution of administered chylomicrons. 2. Nephrotic rats were given intravenous injections of 90 mg of normal rat serum albumin. This did not alter rate of removal of labeled chylomicrons from the circulation. 3. When nephrotic rat plasma was added to *in vitro* clearing system, consisting of normal rat plasma, normal rat chylomicrons and normal rat post-heparin plasma, it significantly decreased rate of clearing. Chylomicrons obtained from normal and nephrotic rats were cleared equally well by serum lipoprotein lipase. 4. Injection of nephrotic rat plasma into normal rats did not alter rate of removal of labeled chylomicrons.

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Rapid Adsorption of Vaccinia Virus on Tissue Culture Cells by Centrifugal Force.* (25767)

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Because of their large size, vaccinia virus particles diffuse slowly. The several hours generally allotted to adsorption of a virus inoculum to cells in monolayer can be reduced if adsorption takes place in suspensions in which the cells are quite concentrated(1). However, difficulty is still encountered in attaining adsorption of a high percentage of the virus in a short time. If accurate measurements of the time between adsorption of a

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virus particle to a cell and some subsequent result are to be made it is necessary to know the moment of adsorption rather than only that it must have occurred at some time during a several hour period. In this paper a method employing centrifugal force is described, which brings cells and virus quickly together in a monolayer.

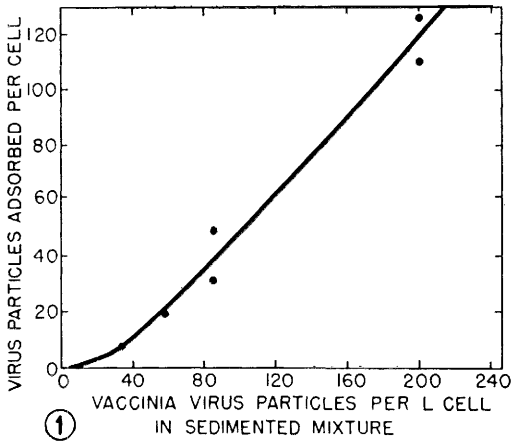
Materials and methods. Vaccinia virus, strain WR, from American Type Culture Collection, adapted to growth in L cells(1) was used in this work. Cells containing 2000 to 3000 virus particles each were stored at −60°C. From this pool, starting virus for the experiments was obtained by breaking the cells with sonic waves(1) to produce suspensions of about 10^9 particles per ml. L cells were grown in a medium containing Earle's balanced salt solution, horse serum and yeast extract(1). Suspensions were made by scraping cells from the glass surface and agitating them by rapid pipetting. Cell counts were made in a hemacytometer. Rapid adsorption of virus to cells is obtained by sedimentation of a suspension of the two in a horizontal tube centrifuge rotor. It is necessary that cells and virus sediment vertically upon a smooth flat surface. This can be produced in the bottom of an ordinary test tube by pouring in about 1 ml of warm melted agar and allowing it to cool. To insure complete freedom from curvature due to meniscus effects at the edges, this cooling is done with the tubes spinning in the centrifuge. The centrifuge rotor must be the horizontal (swinging bucket) type to minimize deposit of material on the walls of tubes. The horizontal, Field-Aligning rotor manufactured by Ivan Sorvall, Inc. of Norwalk, Conn., was used. It has the added advantage, for this work, of double pivots in tube mountings. Tubes swing from vertical to horizontal positions as speed is increased; they swing also somewhat behind or in advance of the radial direction during acceleration and deceleration respectively. This feature tends to assure maintenance of strict perpendicularity between collecting surface and sedimenting forces at all times. The mixture of cells and virus was made to contain just enough cells to form a uniform monolayer on the agar surface. The slower sedimenting virus in the mixture will then fall upon this monolayer where it can be held for any desired time. Following sedimentation the monolayer of cells was removed by rapid pipetting (over 90% were recovered) with growth fluid.‡ The centrifuge tubes were cellulose acetate, 16 mm diameter, and 1.0 x 10^6 cells were used in each tube. Volume of the suspension was such that its depth, over the agar receiving surface, was ½ cm. After sedimentation, cells were washed by suspending them in 5 ml growth medium, sedimenting in pointed tubes, resuspending in a like volume and sedimenting again. Virus was released by exposing the washed cell suspension to 9 kc sonic waves for 2 minutes (1). Virus particles were counted by the sedimentation procedure(1,2) employing electron microscopy and these will be called adsorbed virus.

Results. A series of mixtures of L cells and stock virus was made in 1 ml quantities of growth medium. Each contained 1.0 x 10^6 cells/ml and virus particles in the following concentrations: 34, 58, 85, and 202 x 10^6/ml. These were placed in the swinging bucket centrifuge tubes and the rotor brought up to 8000 rpm (about 8000 x G) in such a manner that increase of speed/unit time was constant. Acceleration time was 1½ minutes. After resuspension and washing of cells, which took an average of approximately 6 minutes, the adsorbed virus was released and counted as described in materials and methods. The results are shown by the graph (Fig. 1) of adsorbed virus particles plotted as a function of virus concentrations in the original mixture.

Preliminary experiments have shown that some of this attached virus is easily eluted from the cells and that new virus first appears, when the multiplicity is high, in about 6 hours from time of inoculation. The cells have shown no evidence of reduction in virus producing ability because of their exposure to the high centrifugal force.

Discussion. When centrifugal force is used to bring cells and virus in contact, the number of virus particles adhering to each cell, after the washing process, is very high. To a

‡ PBS is unsatisfactory for this purpose because with it, cells tend to stick to glass surface.



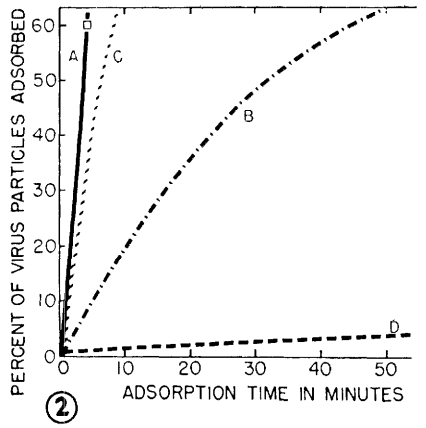


FIG. 1. Vaccinia virus particles adsorbed on L cells by sedimentation from suspensions of various virus concentrations. No. of cells was constant.

FIG. 2. Adsorption of vaccinia virus to L cells when aided by centrifugal force (A) compared with calculated ideal rate for collisions in same suspension (B), ideal rate for a suspension of 5 times greater cell concentration (C), and observed rate for the latter (D).

first approximation, the relationship between particles adsorbed and concentration of the inoculum is linear (Fig. 1). There is no evidence of saturation of cells with virus. There is, however, a noticeable tendency for the line to approach zero in a curve. This tendency is quantitatively the same as that observed in similar experiments using conventional inoculation methods(1). The centrifugal method was designed to bring cells and virus quickly into close proximity so that measurements of the time interval before significant events in infection and growth sequence could be more exact. This it does well as can be seen from the chart, Fig. 2. Calculation based upon the known sedimentation properties of vaccinia virus yields 4½ minutes as the time required for all particles to reach the cell layer. At a multiplicity of 202 (Fig. 1) 60% of the inoculum was adsorbed by the cells (curve A, Fig. 2). If this mixture had been allowed to stand and each collision between virus and cell had resulted in adsorption the calculated adsorption process would have followed curve B.§ This control was not made but one using 5 times as many cells per ml has shown(1) rate of adsorption depicted in curve D. Calculated theoretical maximum for this system would be the rate shown in curve C. Attachment of virus to cells is thus somewhat over 100 times faster than that observed even in suspensions of high cell concentration with a multiplicity of 202.

Summary. By sedimenting a mixture of L cells and vaccinia virus upon a flat surface, 60% adsorption is attained in 4½ minutes. These data have been obtained by destruction of washed cells by sonic waves and counting released virus by electron microscopy.

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§ Calculations based on general equations of Allison and Valentine(3).

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