1. Holden, M., Adams, L. B., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 364.
2. Grossfeld, H., *Science,* 1958, v127, 148.
3. Grossfeld, H., Ragan, C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 63.
4. Franklin, A. E., Sinclair, J. C., *Can. J. Microbiol.,* 1959, v5, 567.
5. Kline, I., Leighton, J., Belkin, M., Orr, H. C., *Cancer Res.,* 1957, v17, 780.
6. Grossfeld, H., *Nature,* 1958, v182, 35.
7. Fisher, T. N., Fisher, E., Jr., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 780.
8. Puck, T. T., Cieciura, S. J., Robinson, A., *J. Exp. Med.,* 1958, v108, 945.
9. Seifter, S., Dayton, S., Novic, B., Muntwyler, E., *Arch. Biochem.,* 1950, v25, 191.
10. Fisher, T. N., Fisher, E., Jr., *Bact. Proc.,* 1960, 123.

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## Effect of Antiserum on Adsorption of Vaccinia Virus to Earle's L Cells.* (26438)

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Neutralized virus is, by definition, not measurable by conventional titration methods. For this reason it has been difficult to assess the part played by altered host cell-virus particle adsorption in the overall neutralization of virus by antiserum. Recent work(1) with Newcastle disease virus, carrying radioactive label, has shown a blocking of adsorption of neutralized virus to host cells. A more direct demonstration of this vital step in the infectious sequence is desirable.

We have described(2) the methods for counting virus particles released by sonic energy from host cells shortly after adsorption. This paper contains preliminary results of application of these methods which show that a substantial part of the neutralization of the infectivity of the virus for L cells is manifest at the point of adsorption.

*Materials and methods.* Vaccinia virus, WR (mouse neurotropic) strain was obtained from the American Type Culture Collection, passed 18 times in L cells and stored at −70°C. L cells were obtained from Dr. W. Earle's laboratory. Medium and other details of the cell cultures have been described(3). Antiserum was produced by vaccination of young New Zealand white rabbits with active virus. Further applications of live virus were made 5 and 12 weeks later with the last application resulting in a typical immune response. Blood was drawn at the 13th week and serum was stored at −20°C. Normal serum was obtained by cardiac puncture from rabbits before vaccination. All sera were heated 30 minutes at 56°C before use. Counting of virus particles was done by the agar sedimentation process (4) employing the electron microscope.

*Experimental.* Mixtures of active virus and serum were made in growth medium such that they contained $10^9$ virus particles per ml and a serum dilution of 1/5. These were incubated 4 hours at 37°C with occasional agitation. L cells were counted, sedimented in pointed centrifuge tubes and resuspended in the various serum-virus mixtures in such quantity that final concentration was $5 \times 10^5$ per ml. There were thus 200 virus particles per cell. Adsorption proceeded at 37°C with frequent agitation for 1½ hours, after which the cells were washed twice and the remaining adsorbed virus released by 2 minutes treatment with 9 KC sonic energy. This cell-

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TABLE I. Effect of Antiserum in Reducing Adsorption of Vaccinia Virus to L Cells.

| Serum in medium | L cells/ml suspension | Virus particles released/ml of sonic treated, washed cells | Virus particles/cell |
|---|---|---|---|
| Normal rabbit | $3.0 \times 10^5$ | $3.6 \times 10^6$ | 12 |
| Immune " | $3.7 \times 10^5$ | $<3.6 \times 10^5$ | <1 |

associated virus was then counted.

Results of a typical experiment are shown in Table I. An average of 12 virus particles was found per L cell when adsorption of active virus proceeded in presence of normal serum, while none were found when neutralized virus was used in presence of antiserum. This encouraging result led to further experiments where adsorption could be increased and the results expressed in larger and, therefore, more statistically significant numbers.

Sedimentation adsorption experiments were made with the same serum-virus-cell mixtures as those above. Immediately after addition of cells the mixtures were sedimented at 8000 G for 7 minutes, depositing both cells and virus in a thin layer on a smooth agar receiving surface(5). Cell density on this surface was $5 \times 10^5$ per $cm^2$.

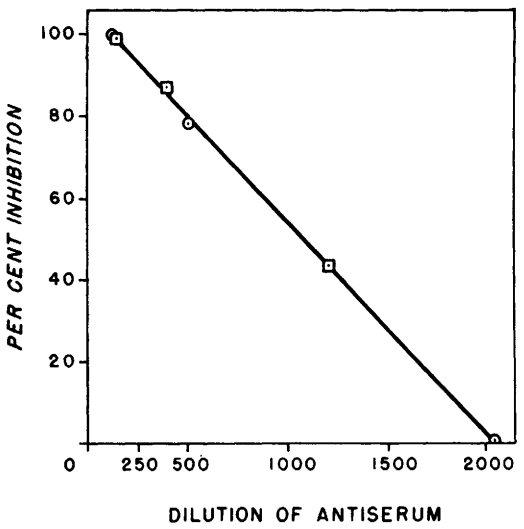


FIG. 1. Neutralization of vaccinia virus by rabbit antiserum as measured by plaques on L cell monolayers. % inhibition is:

$$100\left(1-\frac{\text{No. of plaques with antiserum dilution}}{\text{No. of plaques with normal serum dil.}}\right).$$

After resuspension, washing and sonic treatment of the cells, the adsorbed virus was released by sonic treatment and the particles counted. Of the 200 virus particles available per L cell, an average of 70 was found adsorbed in presence of normal rabbit serum and 60 with normal horse serum. With immune rabbit serum, only 14 particles were found per cell (Table II). These data are typical of this reaction, showing that 80% of the virus adsorption which proceeds in normal rabbit serum is prevented by the immune serum of a dilution of 1-5. The small difference in results with normal horse and rabbit sera is probably not significant.

TABLE II. Effect of Antiserum in Reducing Adsorption of Virus to Cells Even When Contact Is Increased by Centrifugal Force.

| Serum in medium | L cells/ml suspension | Virus particles released/ml of sonic treated, washed cells | Virus particles/cell |
|---|---|---|---|
| Normal rabbit | $4.7 \times 10^5$ | $3.3 \times 10^7$ | 70 |
| " horse | $5.5 \times 10^5$ | $3.3 \times 10^7$ | 60 |
| Immune rabbit | $4.7 \times 10^5$ | $6.8 \times 10^6$ | 14 |

Evaluation of the antiserum was made by titrating residual virus activity on monolayers of L cells. Equal amounts of virus were added to serial 3X dilutions of serum and the mixtures held at 37°C for 30 minutes. A 0.1 ml sample of each dilution was inoculated, in duplicate, onto monolayers in 1 ounce prescription bottles. These bottles were incubated at 37°C for 3 hours with frequent tilting to allow for even distribution. The monolayers were then washed with Hank's BSS and overlaid with 1% Noble agar in nutrient fluid. On the third day a second overlay containing neutral red (1:50,000) was added. Controls consisted of untreated virus and virus treated with normal rabbit serum.

Results of 2 separate titrations are plotted in Fig. 1. Cultures receiving immune serum dilution about 1-1000 showed half as many plaques as controls with normal serum.

*Discussion.* Although much has been written about neutralization of virus by specific antiserum, most of the comment is highly speculative regarding the part played by an-

tibody in the process of adsorption of virus to host cells. Hulten and McKee(6) have suggested the existence of 3 zones of neutralization of influenza virus. In the first of these some virus propagation can still proceed; in the second this is stopped but virus fixation to the cell still occurs; while in the third, "probably" fixation of virus to the cell is also blocked. Rubin and Franklin(1) working with radioactive NDV conclude, somewhat similarly, that although one antibody molecule per virus particle will not block all particle adsorption, it does block infection. They believe several molecules of antibody are necessary to prevent adsorption of the virus particle to the cell.

The data presented here show with singular directness that 4/5 of the expected adsorption of vaccinia virus to susceptible L cells was blocked by antiserum. During these experiments, washing of the cells was sufficient to reduce the amount of unattached virus below the counting level. Virus and cells were brought into close proximity by centrifugal force. In the experiments involving ordinary mixing, no adsorption was observed. It must be remembered, though, that counting of virus particles at the level of one per cell is difficult. This experiment shows only that adsorption is blocked by antibody at least as much and possibly more than in the one employing centrifugal force. Work is continuing to determine whether quantity of antibody required to cause a given amount of adsorption inhibition is determined by total number of virus particles or by number of infectious units.

*Summary.* Antivirus rabbit serum is shown to block 80% or more of the adsorption of vaccinia virus to susceptible L cells. Data supporting these conclusions are provided by direct virus particle counts with the electron microscope.

1. Rubin, Harry, Franklin, R. M., *Virology,* 1957, v3, 84.

2. Smith, K. O., Sharp, D. G., *ibid.,* 1961, March.

3. ———, *ibid.,* 1960, v11, 519.

4. Sharp, D. G., *Proc. 4th Int. Conference on Electron Microscopy,* Berlin, 1958, 542.

5. Sharp, D. G., Smith, K. O., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 167.

6. Hultin, J. V., McKee, A. P., *J. Bacteriol.,* 1952, v63, 437.

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