MEASUREMENT OF INFLUENZA VIRUS-ANTIBODY REACTION
BY QUANTITATIVE ELECTRON MICROSCOPY

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ABSTRACT

Measurement of the weight of individual virus particles from untreated and antibody-treated populations was made by quantitative electron microscopy. The weight of antibody bound depended on the concentration of antibody in solution. One population of viruses exposed to an antibody concentration which resulted in 95% inhibition of hemagglutination showed a mass increase of 55%, corresponding to an absolute increase of $9.0 \times 10^{-17}$ g in the median value. Another population, whose hemagglutination inhibition assay was 64%, showed a 39% increase in mass corresponding to an absolute median increase of $7.3 \times 10^{-17}$ g. The larger viruses in each population bound a greater absolute amount of antibody than did the smaller ones, but the latter bound relatively more antibody in proportion to their mass. No cross-reactivity was found between the antibody to influenza A/PR8 and the influenza strain B/LEE. Influenza A/PR8 controls exposed to nonspecific gamma-globulin displayed a significant weight loss, at least in part owing to loss from the core, as judged from the electron micrographs.

INTRODUCTION

A method of quantitative electron microscopy that permits the measurement of mass to $10^{-18}$ g has been developed through the work of Zeitler and Bahr (8), Lenz (6), Hall (4), Burge and Sylvester (2), and others. In this study the method of Zeitler and Bahr was used with minor modifications to quantitate the reaction, on an intact biological object, between a known antigen and its antibody. The influenza virus was chosen as a model whose physical and serologic properties have been intensively studied. The sensitivity of previous methods used to quantitate antigen–antibody reactions has been given by Haurowitz (5) as 12–100 μg antibody by the microprecipitation technique and 0.03 μg both by passive hemagglutination and Coombs antiglobulin tests.

MATERIALS AND METHODS

Preparation of Virus

Viruses of the influenza strains A/PR8 and B/LEE were grown in embryonated eggs and purified by adsorption to, and elution from, human type “O” cells; this group of viruses will be referred to as population A. A second lot of A/PR8 virus, prepared similarly, was banded in a sucrose density gradient (12–42%) to produce a more uniform population, referred to as population B (Fig. 1).
**TABLE I**

Reaction of Influenza A/PR 8 with Specific and Nonspecific Antibodies and Correlation of Antibody Uptake with Hemagglutination Inhibition Assay

<table>
<thead>
<tr>
<th>Virus population</th>
<th>Mean No. of Virus Particles X 10^12 g</th>
<th>No. Virus/globulin</th>
<th>HI assay*</th>
<th>Mass change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td><strong>A</strong> A/PR8 lot 67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>95</td>
<td>16</td>
<td>5.3</td>
<td>+55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-V-PR8</td>
<td>119</td>
<td>25</td>
<td>6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B</strong> A/PR8 lot 72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>118</td>
<td>19</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plus anti-V-PR8</td>
<td>104</td>
<td>26</td>
<td>3.9</td>
<td>0.03/0.36</td>
<td>+39 &lt;0.001</td>
</tr>
<tr>
<td>Plus nonspecific</td>
<td>104</td>
<td>16</td>
<td>3.8</td>
<td>0.03/0.40</td>
<td>-16 &lt;0.001</td>
</tr>
<tr>
<td><strong>C</strong> A/PR8 lot 72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>95</td>
<td>16</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plus nonspecific only</td>
<td>100</td>
<td>12</td>
<td>2.3</td>
<td>-25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plus nonspecific and Sephadex</td>
<td>97</td>
<td>10</td>
<td>2.5</td>
<td>-38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>D</strong> B/LEE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>156</td>
<td>16</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plus nonspecific</td>
<td>132</td>
<td>16</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plus anti-V-PR8 and Sephadex</td>
<td>93</td>
<td>15</td>
<td>3.2</td>
<td>-6.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Hemagglutination inhibition assay
† Nonspecific globulin

**Preparation of Antibody and Reaction with Virus**

Antibody was produced in guinea pigs to the "V" antigen, i.e. the specific strain antigen of the virus, which had been prepared by the ether method (7). Gamma globulin was isolated, by the rivanol procedure, from the hyperimmune serum and excess rivanol was removed by passage through Sephadex G-50 (3). This globulin was used as specific antibody, "anti-V-PR8." Nonspecific antibody was prepared as the ethanol fraction II of normal guinea pig gamma-globulin.

Both populations of influenza A/PR8 and one of influenza B/LEE were reacted with anti-V-PR8, and unbound globulin was almost totally removed by three sedimentations of the virus-antibody complexes at 30,000 g followed by resuspension in 0.1 m phosphate buffer, pH 7.0. Additional samples from the three populations were reacted with nonspecific antibody and were washed as before. In an effort to obviate the significant loss of mass observed after the latter procedure, further samples of A/PR8 and B/LEE were reacted with nonspecific antibody, and all unreacted protein was removed from the virus on Sephadex G-200.

Antibody nitrogen was measured by a micro-Kjehldahl procedure. Hemagglutination-inhibition assay, with human type O cells, was employed as an independent estimate of antibody uptake. The test employed four hemagglutinating units of antigen and dilution volumes of 0.25 ml (Table I).

**Measurement of Mass**

Formvar-carbon-coated grids were placed on droplets of virus suspension, washed three times, 5 min each, on droplets of 0.1 m ammonium acetate and then were dried in air. Polystyrene latex spheres, approximately 1260 A in diameter, were sprayed on the grids for use as focusing aids and weight standards. Magnifications were measured by means of a diffraction grating replica (21,000 lines/cm). Photographs were taken at a magnification of approximately 50,000 times with a Hitachi HU 11-A electron microscope equipped with a special photometer-exposure timer which permitted exact exposures necessary to produce the required background densities. Other operating conditions included an accelerating voltage of 50 kv; objective aperture, 30 μ; liquid nitrogen in the specimen-stage cold trap; and the use of the high-contrast specimen holder. Photographs were made as close to focus as possible to obviate the effects of phase contrast which had a significant influence on the measurement of mass of these small particles.
Figure 1  Influenza A/PR8 virus, negatively stained with 2% phosphotungstic acid, pH 6.4. A range of sizes is evident in the population. Distortions of some of the virions were not seen in unstained preparations, × 100,000.
The negative images of the virus particles (Figs. 2, 3) and the latex spheres were scanned in a Jarrell-Ash recording microdensitometer and the areas under the resulting curves were measured with a compensating polar planimeter. The mass of each sphere was calculated from its known density (1.05) and its radius, which was derived from the measured image. A factor relating area under the curves to mass was derived from the spheres, and the mass of individual virions was determined. Prolonged exposure to the beam, even with the use of liquid nitrogen, led to contamination of the spheres which resulted in progressive decrease in the factor.

Each population was plotted linearly on probability paper as percent integral frequency against logarithm of weight (1).
in mass after exposure to nonspecific globulin is a constant fraction of the original virus mass and seems to involve loss of substance from core as well as surface. The mechanism of the loss has not been elucidated. However, it was determined that unreacted gamma-globulin could be removed more conveniently and efficiently by passage through a Sephadex G-200 column than by the sedimentation method usually employed for this purpose.

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For References, see page 66.

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Weight loss in PR8 after exposure to nonspecific gamma globulin

0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40
Log weight (g x 10^-17)

Per cent integral frequency

- A/PR8 lot 72-untreated
- A/PR8-gamma globulin only
- A/PR8-gamma globulin+Sephadex

FIGURE 5 The effect on influenza A/PR8 of exposure to nonspecific gamma-globulin. This loss of mass was not reflected in a change in the hemagglutination inhibition assay.

REFERENCES
Figure 6  Influenza A/PR8, population B, treated with nonspecific gamma-globulin, negatively stained. X 100,000.

Figure 7  Influenza A/PR8, population B, treated with anti-V-PR8, negatively stained. X 100,000.