A Serologic Survey of Influenza and APC Virus Infections

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As the authors point out, the study reported upon here will serve as a base line upon which findings of similar surveys in years to come may be judged. We think many epidemiologists will be interested in establishing their own comparable base lines.

The present study is the third undertaken in the division to learn whether the routine blood specimens which steadily flow into the laboratory carry useful epidemiologic information. The first study was made in the winter of 1945-1946. More than 300 pools of five sera each were periodically collected and tested with allantoic fluid influenza antigens. The results suggest that little influenza A virus infection had occurred during the winter, but that sporadic epidemics of influenza B virus infection had been present. High titers of influenza B antibody were found following reports of outbreaks of acute undifferentiated respiratory infections during December.

The second study, in 1952–1953, was made in anticipation of a predicted epidemic of influenza A. Sporadic outbreaks evidently did occur and the randomly collected sera again gave consistent results. Elevated influenza A antibody titers were frequently encountered. In this study soluble antigen replaced allantoic fluid and the sera were examined individually. Both of these changes were advantageous.

The present study covers the winter of 1954–1955. In addition to influenza A and B, the specimens were tested for complement-fixing antibodies for the APC (adenoidal - pharyngeal - conjunctival) group of viruses.

Materials and Methods

The quantitative complement-fixation test devised by Wadsworth and Malaner was used, with the modifications made in the 1952–1953 study. The specimens were portions of sera that had been submitted for tests for syphilis and had not reacted with cardiolipin antigen. Collections were made to represent the eight regions used in the original survey. In all, 388 sera were tested. Each was first screened in a 1:4 dilution with the three antigens and reacting sera were retested to determine the titers. The fixation period was 24 hours at from 3°C to 6°C for both tests.

The APC antigen consisted of infected tissue culture (HeLa) fluids. Two modifications were introduced. The virus was left on the cells for a longer period and the contents of the bottles were quickly frozen and thawed. A pool composed of aliquots of APC antigen Types 1–6 was used in the serologic tests. The antigen was inactivated for 30 minutes at 56°C each time before it was used.

Standardization of Antigen—The range of activity of the antigen was determined with an immune serum of known activity. The optimum dilution was that which gave the highest titer in the absence of any anticomplementary

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Table 1—Titration for Linearity Between Serum and Complement Performed with APC Antigen and Immune Serum

<table>
<thead>
<tr>
<th>Immune Serum Dilutions (0.05 ml each)</th>
<th>Equivalent ml of Serum</th>
<th>APC Antigen Dilutions in Optimum Range</th>
<th>Maximum No. of Units of Complement Required for 50 Per cent Hemolysis (^{12b})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:2  1:4  1:8  1:1.33  1:2.67  1:5.33 undil. 1:2  1:4  1:1.33 1:2.67</td>
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<tr>
<td>1:5.55</td>
<td>0.009</td>
<td>0 10 75 25 30 75</td>
<td>10 100</td>
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<tr>
<td>1:8.33</td>
<td>0.006</td>
<td>0 10 75 25 30 75</td>
<td>30 90</td>
</tr>
<tr>
<td>1:16.7</td>
<td>0.003</td>
<td>0 10 75 25 30 75</td>
<td>70 100</td>
</tr>
<tr>
<td>1:25</td>
<td>0.002</td>
<td>0 10 75 25 30 75</td>
<td>90 100</td>
</tr>
<tr>
<td>1:33.3</td>
<td>0.0015</td>
<td>0 10 75 25 30 75</td>
<td>90 100</td>
</tr>
<tr>
<td>1:41</td>
<td>0.0012</td>
<td>0 10 75 25 30 75</td>
<td>70 100</td>
</tr>
<tr>
<td>1:50</td>
<td>0.001</td>
<td>0 10 75 25 30 75</td>
<td>4.5</td>
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<tr>
<td>1:55.5</td>
<td>0.0009</td>
<td>0 10 75 25 30 75</td>
<td>4.1</td>
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<tr>
<td>1:62.5</td>
<td>0.0008</td>
<td>0 10 75 25 30 75</td>
<td>4.1</td>
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<td>1:83.3</td>
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<td>0 10 75 25 30 75</td>
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<td>1:100</td>
<td>0.0005</td>
<td>0 10 75 25 30 75</td>
<td>3.2</td>
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Properties. Dilutions which gave less than 90 per cent hemolysis with two 50 per cent units of complement in the absence of immune serum were considered unsatisfactory.

Linearity between serum and complement was determined by using proportionally increasing amounts of serum and antigen with varying amounts of complement. The dilutions were chosen on the basis of the range test just described. An example is shown in Table 1. Figure 1 represents the titration. The dilution already chosen as optimum for three 50 per cent units and those for four, five, six, and nine units gave the maximum degree of reaction. The linearity obtained indicates that the conversion factors determined for the complement-fixation test for syphilis apply fairly well to the APC antigen.  

Tests were then made with three 50 per cent units of complement and three dilutions of the antigen in the optimum range with serial twofold dilutions of sera from patients with respiratory symptoms. The amount of antigen producing maximum fixation may vary with different human sera. The average optimum dose (1:4) was used. This amount may be expected to give titer values within 25 per cent agreement when calculated from partial points of hemolysis obtained with different amounts of serum. Excess or deficient amounts of antigen may give atypical results and usually do not produce the maximum titer. Thirty-one sera were tested with three amounts of APC antigen. The titers ranged from seven to 60 with the exception of one which was greater than 200. This patient had served at Fort Dix where Dr. Hilleman had conducted his studies of military personnel. His serum was used as a positive control.

Results

The data are shown graphically in Figure 2. On the basis of our experience in the first two surveys we assume that influenza A and B virus infections were infrequent during the period of observation. The titers obtained probably represent residual antibodies developed during subclinical or previous infections. The presence of APC virus antibodies in high titer suggests that infection with these agents was most prevalent. During February 80 per cent of the sera reacted strongly with the APC antigen and in approximately one-half the titers were greater than 50. Since we have thus far had little opportunity

**Figure 1**—Complement-Serum Relationship APC Antigen

**Figure 2**—Range of Complement-Fixation Titers in Sera Collected from December, 1954—April, 1955
to determine the degree of antibody response following proved infections, the significance of particular titers is uncertain although the changes seem to be meaningful.

Figure 3 shows the eight regions of the state. In each case reacting sera were widely distributed.

Discussion

The epidemiologic value of surveys of this kind remains to be proved. So far the results have conformed rather well with clinical information. What has been done provides a base line which may be helpful in judging results in other years. While the sera are randomly selected they are drawn from an arbitrarily determined group, namely, young, healthy adults whose sera come to the division in a constant flood for purposes of employment or premarital testing. This group has been selected to avoid the complexities of hospitalized patients and because the source of material is large and most nearly uniform. The possible value of such sampling may more likely become clear from continued experience than from closer analysis.

Sporadic cases of influenza A infection seem to have been commonplace in each of the three years. Neither influenza A nor B has been as prevalent as APC virus infections seem to have been during the winter of 1954–1955.

Conclusions

A third survey for respiratory virus antibodies in random New York sera has provided evidence of sporadic influenza A and influenza B virus infections. APC virus antibodies were more frequently found.

The sera sent to public health laboratories, though not representative of the population of given regions, provide means for learning something about the prevalence of respiratory virus infections.

The quantitative complement-fixation test has been applied to the APC virus system.

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REFERENCES