ELECTROPHORESIS OF PURIFIED ANTIBODY PREPARATIONS

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In connection with a study of the ultracentrifugal sedimentation of antibody preparations (1) the opportunity arose for study of the electrophoretic properties of some of the highly active material under investigation. Measurements of this kind are of value in the characterization of proteins and similar high molecular substances, and may also give information regarding the chemical homogeneity of the material, as shown in previous publications (2a).

The measurements given in the present paper form part of a more detailed, as yet uncompleted investigation of the electrochemical properties of proteins in normal and immune sera. Since, however, the ultracentrifugal study referred to above was made on the same material, it was thought advisable to publish the electrophoretic data at the present time.

Of the material used in the work of Heidelberger and Pedersen the following was investigated.

From Horse Sera.—Preparation 1: Obtained by dissociation of a Type I anti-pneumococcus specific precipitate with 15 per cent sodium chloride; 61 per cent of the total nitrogen was specifically precipitable ((1), Experiment 19).

Preparation 2: Obtained by the same dissociation method applied to the specific precipitate from a Felton solution from Type I antipneumococcus serum; 87 per cent of the nitrogen was specifically precipitable. This corresponds to the solution used in (1), Experiment 21.

Preparation 3: Dissociated Type I pneumococcus anticarbohydrate, obtained from unpreserved serum ((1), Experiment 18). More than 51 per cent of the nitrogen present was specifically precipitable.

From Rabbit Serum.—Preparation 4: Dissociated Type III pneumococcus anticarbohydrate. 90 per cent of the nitrogen content was specifically precipitable ((1), Experiments 7 and 8).

The method for the electrophoresis measurements (ultraviolet photography of the moving boundaries) has been described in previous publications (2a, b).
rather low potential gradient must be used on account of the high conductivity of
the electrolyte media, which, for investigation of serum globulin must usually
have a rather high concentration to keep the substance in solution. Preparation
4 furnishes an exception, being soluble in distilled water. Thus an ionic strength
of \( \mu = 0.1 \) was used in all buffer solutions (acetate and phosphate) except those
for the last mentioned preparation, for which \( \mu = 0.02 \) was used. The quantities
available were rather small, usually sufficient for only one determination. How-
ever, it was possible to recover most of the material at the conclusion of each
experiment by applying gentle suction to the capillary tube at the bottom of
the U tube. The recovered solution was then dialyzed against the buffer to be
used in the next experiment. The protein concentration was 0.2 per cent.

The results are collected in Table I, and in Figs. 1 and 2. For com-
parison the mobility-pH curves for normal serum globulin from horse
and rabbit sera (which are practically identical) have been plotted
as broken lines in the corresponding diagrams (3).

Only preparation 3, which had been obtained from a serum contain-
ing no preservative, showed homogeneous electrophoresis. This
material was also quite homogeneous in the centrifuge. However,
too much stress should not be laid upon this result as far as elec-
rophoresis is concerned. The voltage in these experiments is very low
compared with that used in other cases (water soluble proteins) and
some inhomogeneity might have escaped detection. It is therefore
remarkable that the other three preparations showed a very pro-

### Table I

Electrophoretic Mobilities of Antibody Preparations at 20.0°C. in Acetate and
Phosphate Buffer Solutions of Varying pH and Constant Ionic Strength

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>Mobility in cm.² volt⁻¹ sec.⁻¹ ( \times 10^4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preparation 1</td>
</tr>
<tr>
<td>Acetate</td>
<td>4.63</td>
<td>+3.9</td>
</tr>
<tr>
<td>Phosphate</td>
<td>5.24</td>
<td>+1.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>5.30</td>
<td>+1.8</td>
</tr>
<tr>
<td>&quot;</td>
<td>5.47</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>6.23</td>
<td>-0.9</td>
</tr>
<tr>
<td>&quot;</td>
<td>6.96</td>
<td>-2.8</td>
</tr>
</tbody>
</table>

Isoelectric point: pH = 5.94  
Slope of curve at isoelectric point: \( \frac{du}{dpH} = 2.7 \times 10^{-4} \)  

\( \frac{du}{dpH} = 3.9 \times 10^{-4} \)
nounced inhomogeneous migration. Such is also the case for serum globulin, as has been shown (3). While no indication of definite components could be observed in serum globulin, the antibody preparations 1 and 2 and possibly also 4 showed the presence of appreciable amounts of a substance migrating at a rate quite different from that of the bulk of the material. Its mobility could be calculated at pH 5.30 and pH 7.34, as $-1.3 \times 10^{-4}$ and $-6 \times 10^{-4}$ cm$^2$ volt$^{-1}$ sec$^{-1}$, respectively. This component therefore has a much more acid iso-electric point, and it is to be noted that the mobilities found would fit fairly well into the curve for preparation 3, the homogeneous antibody from horse serum.

The rabbit serum preparation 4 gave much more diffuse boundaries on account of its higher diffusion, and did not allow similar calculations to be made with any degree of certainty.

Fig. 1. Mobility of antibody preparations from horse serum at different pH (temperature 20.0°C.). O, preparation 1; □, preparation 2, Δ, preparation 3. Broken curve, normal horse serum globulin.
If the acid component in preparations 1 and 2 is really identical with the homogeneous preparation 3, the bulk of the material in the inhomogeneous preparations would probably be denatured antibody. These solutions were also found to be very inhomogeneous in the ultracentrifuge. Pedersen has found that denaturation may be accompanied by a considerable shift in the isoelectric point toward the alkaline side (4). It would, however, still be necessary to account for the finding that of the two last mentioned preparations No. 2 has a higher specific precipitability and No. 1 about the same as that of the homogeneous preparation 3.

At any rate the results as shown in Figs. 1 and 2 demonstrate that the antibody preparations are distinctly different from whole serum globulin. For all three horse serum preparations the low mobility at alkaline pH is characteristic. Evidently the number of ionized groups is unusually small. The rabbit antibody preparation differs

![Graph showing mobility of an antibody preparation (No. 4) from rabbit serum at different pH (temperature 20.0°C.). Broken curve, normal rabbit serum globulin.](image-url)
strikingly from normal rabbit serum globulin in its isoelectric point, which is especially noteworthy as these substances could not be distinguished in the ultracentrifuge. We have here one of the numerous instances of identical sedimentation but different electrochemical properties.

Of course, one may raise the objection that the observed differences between antibody and normal serum globulin are due to changes taking place in the course of preparation, although very mild methods have been applied. However, in some recent experiments with an improved apparatus (5) we have been able to show that normal and immune serum, which have been exposed only to dialysis against a buffer solution, show the presence of several differently migrating globulins when subjected to electrophoresis at high voltage. In immune sera the antibody function is found only in one of these fractions, namely one of the most slowly migrating components, in agreement with the results obtained above. A considerable purification of antibodies could be obtained in this way in horse as well as in rabbit sera (6).

**SUMMARY**

Electrophoretic mobilities of antibody preparations isolated from type specific antipneumococcus horse and rabbit sera, measured over a range of pH values, show that these preparations are distinctly different from normal serum proteins in their electrochemical properties.

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**BIBLIOGRAPHY**
