THE EFFECT OF ACETIC ACID ON THE STABILITY OF SERUM PROTEINS*

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Several investigators (1, 2) have shown that when horse serum is denatured a change in the relative distribution of electrophoretic components occurs. Van der Scheer, Wyckoff, and Clarke (1) found that heating of serum at 65° resulted in the formation of a single component, with the disappearance of the albumin and globulin peaks of the serum. This was confirmed by Davis, Hollaender, and Greenstein (2) who also demonstrated a similar phenomenon produced by ultraviolet radiation.

In the present study the stability of serum and certain plasma protein fractions at acid pH values in the presence of acetate has been investigated. To determine the stability of these proteins, electrophoretic analysis was carried out after removal of the acetate ions and the results are compared with those obtained with undenatured human serum.

EXPERIMENTAL

Usually three or four different samples of normal human serum were pooled and adjusted to the desired pH by dropwise addition of acetic acid with sufficient stirring to minimize any possible effects of local excess of acid. After standing at room temperature for 30 minutes, the solutions were dialyzed at 3–5° against 2000 cc. of 0.15 m sodium chloride for 2 days with several changes of the dialysate. The samples were then diluted with 0.1 N sodium diethyl barbiturate buffer of pH 8.6 to a final protein concentration of 1.87 per cent, i.e. 3.0 mg. of protein nitrogen per cc., and dialyzed in the cold for 3 days against liberal portions of this buffer.

The electrophoretic experiments were performed at 1.5° in a Tiselius apparatus (3) equipped with the schlieren scanning device of Longsworth (4). The concentrations of the electrophoretically separable components were estimated by the procedure of Tiselius and Kabat (5). The results are expressed as the ratio of the area of each component to the total area, ex-

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clusive of the $\delta$- and $\epsilon$-boundaries. Average values of the descending and ascending boundaries are given. Mobilities were computed from the descending patterns and refer to $0^\circ$.

Results

In Fig. 1 are superimposed the tracings of the electrophoretic patterns of normal human serum before and after exposure to acetic acid at pH 3.0 for 30 minutes. Electrophoresis of the acetic acid-treated serum showed a progressive increase in homogeneity of the material with the formation of a new peak. This new component, designated as D (for denatured), migrates with a mobility intermediate to mobilities of the $\alpha_2$- and $\beta$-globulins.

![Fig. 1. Superimposed tracings of a 1.87 per cent solution of normal human serum before and after treatment with acetic acid at pH 3.0. Electrophoresis was carried out in a sodium diethyl barbiturate buffer at pH 8.6 and 0.1 ionic strength at a potential gradient of 5.3 volts per cm. for 12,600 seconds.](image)

Exposure to acetic acid at pH 3.0 for 30 minutes was found to be necessary for the appearance of this component. In samples treated for 30 minutes, the D component has the same mobility as that of a sample treated for 48 hours, although in the latter case the D component is present in a somewhat higher concentration.

Effect of pH—The striking differences of the two patterns of Fig. 1 made it desirable to test the effects of acetic acid at various pH values. Table I shows the apparent distribution of the protein components observed in six experiments in which the pH was varied between 4.0 and 2.8. After exposure to pH 4.0, no significant changes were found. At pH 3.7, the $\alpha_1$-globulin increased at the expense of the albumin. pH 3.4 causes a further shift in the concentration of these two components, and the D component appears. At pH 3.1, the amount of $\gamma$-globulin is reduced. These results indicate clearly that in the case of normal human serum in an ace-
tic acid medium pH 4.0 represents the critical pH for the stability of the proteins. At pH values below 4.0 denaturation occurs.

In a series of experiments in which hydrochloric acid, lactic acid, or equilibration with a glycine-hydrochloric acid buffer was used for adjustment to pH 3.0, the formation of the D component was not observed. In these cases all the electrophoretic components of normal serum were present. However, a slight shift in the distribution of the relative concentrations of components was noticeable which was most marked in the case of the lactic acid-containing medium.

**Stability of Plasma Fractions**—Since it was found that the serum globulins are more specifically affected by the presence of acetic acid, the stability of plasma fractions was investigated. Various preparations were tested. Only in Fraction IV-1, which contains α₁, α₂, and β-globulins, traces of albumin and γ-globulin, and which is characterized by a lipide content of 15.0 per cent, was a change similar to the one in serum noticed. This suggests the lability of lipide-protein linkages as the cause of the D peak. Another observation seems worth recording. In Fig. 2 are shown the electrophoretic patterns of γ-globulin before and after treatment with acetic acid at pH 3.0 for 30 minutes. The average mobility, \( u = -1.2 \times 10^{-8} \text{ cm}^2 \text{ sec}^{-1} \text{ volt}^{-1} \), remains unchanged. The somewhat greater homo-

<table>
<thead>
<tr>
<th>Pretreatment of serum</th>
<th>Concentration in per cent as</th>
<th>Mobilities ( \times 10^{-8} \text{ cm}^2 \text{ sec}^{-1} \text{ volt}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Albumin ( \alpha_1 )</td>
<td>Albumin ( \alpha_2 )</td>
</tr>
<tr>
<td>0.294 4.06</td>
<td>58.3 5.2 10.4 14.0 12.1</td>
<td>-6.4 -5.2 -4.1 -3.0 -1.3</td>
</tr>
<tr>
<td>0.735 3.70</td>
<td>57.1 5.6 12.1 13.2 12.0</td>
<td>-6.4 -5.2 -4.1 -3.0 -1.2</td>
</tr>
<tr>
<td>1.47 3.40</td>
<td>53.1 9.4 14.8 11.0 11.7</td>
<td>-6.3 -5.1 -4.0 -3.1 -1.2</td>
</tr>
<tr>
<td>2.94 3.10</td>
<td>47.0 14.9 28.0 10.1</td>
<td>-6.3 -5.2 -3.7 -1.2</td>
</tr>
<tr>
<td>5.88 2.80</td>
<td>45.0 16.0 34.8 4.2</td>
<td>-6.3 -5.3 -3.6 -1.2</td>
</tr>
</tbody>
</table>

Electrophoresis was carried out in a sodium diethyl barbiturate buffer at pH 8.6 and 0.1 ionic strength.

1 The products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, by the Department of Physical Chemistry, Harvard Medical School, Boston, under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Harvard Medical School.
geneity of the pattern in Fig. 2, B, however, is accompanied by an increase of irreversibly denatured protein which, in the presence of neutral salt, precipitates in the neighborhood of the isoelectric pH of this protein. Moreover, it seems of interest to consider this result in connection with the one described above; namely, that exposure of purified γ-globulin to pH 3.0 in the presence of acetic acid does not alter the mean mobility of the protein

A. Normal

B. Acetic acid treated

Fig. 2. Electrophoretic patterns of human γ-globulin. The patterns were obtained from a 1.0 per cent protein solution in sodium diethyl barbiturate buffer at pH 8.6 and 0.1 ionic strength at a potential gradient of 5.2 volts per cm. for 10,800 seconds.

fraction, whereas the same treatment causes this component to disappear in the serum.

DISCUSSION

It is apparent from the results presented in this paper that marked alterations occur in the distribution of electrophoretic components of serum after exposure to acetic acid for 30 minutes. These changes occur in a relatively short time and are dependent on the pH of the solution. No experiments to give a detailed account of the nature of this particular dena-
turation have been carried out. From the fact that exposure to acetic acid of the purified plasma fractions, e.g. albumin and \( \gamma \)-globulin, does not produce a significant shift of the electrophoretic mobilities, we may assume that the prevailing experimental conditions do not alter markedly the number of ionizable groups of these proteins. In the case of the lipide-rich protein fraction, Fraction IV, however, a change in the electrophoretic behavior was noticed. This leads us to believe that fission of lipide-protein linkages may be the cause of this phenomenon. The splitting of such bonds and a simultaneous recombination of molecules may readily yield aggregates of different size, but with a similar charge distribution such a mechanism could easily explain the formation of the D peak observed in the case of serum. A similar result was obtained by Tiselius and Horsfall in their work on the dissociation and reassociation of different hemocyanins (6).

From a comparison of the effect of acetic acid on serum proteins with that of other acids, i.e. hydrochloric acid, lactic acid, and the glycine-hydrochloric acid mixture, it is apparent that the formation of the D component at pH 3.0 is relatively specific for the presence of acetate ions. It therefore seems worth while to stress that denaturation does not depend on the nature of the protein and the acidity of the medium alone, but that the type of ions present in the medium also plays a rôle in determining the degree of stability of a protein. Moreover, it was noticed that under any given experimental condition the rate of denaturation may be enhanced considerably by the presence of neutral salts.

**SUMMARY**

Exposure of serum to acetic acid at pH values below 4.0 causes an irreversible denaturation of the proteins. This phenomenon is dependent on the pH of the solution. At pH 3.0, a new electrophoretic component, designated as D, was observed, which migrates with a mobility intermediate to mobilities of the \( \alpha_2 \)- and \( \beta \)-globulins.

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