

## ELECTROPHORETIC SEPARATION OF SKIN-SENSITIZING ANTIBODY FROM THE SERA OF RAGWEED-SENSITIVE PATIENTS\*

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THE skin-sensitizing antibody has been found to be associated with many of the common forms of allergy. It differs from precipitating antibody in that it is more heat labile and does not produce a precipitate with its specific antigen *in vitro*. It is usually detected by means of skin testing procedures and it can be transferred by the Prausnitz-Küstner test, using whole serum. Precipitating antibodies appear to be present in the gamma globulin fraction of sera. On the other hand, the localization of skin-sensitizing antibody to any particular protein fraction of sera is not well established.

Scherrer,<sup>1</sup> using salting out methods with ammonium sulfate and sodium sulfate, was able to show that skin-sensitizing antibody in sera of patients sensitive to grasses, ragweed, and egg white was mainly present in the pseudo-globulin fraction, partly in the euglobulin fraction, and absent from the albumin fraction. Using a similar method, Stull, Sherman, and Cooke<sup>2</sup> demonstrated that the skin-sensitizing antibody in sera of patients sensitive to ragweed was associated with the pseudo-globulin fraction. Newell and associates<sup>3</sup> used the Tiselius electrophoresis apparatus to separate the sera of patients sensitive to ragweed and rabbit dander, and were able to show that sensitizing antibody was present in the gamma globulin fraction but not in the beta globulin or albumin fractions.

Using electrophoretic convection methods of studying allergic serum, Campbell and his associates<sup>4, 5</sup> have reported skin-sensitizing activity in the alpha and beta globulin fractions in one case. Cooke and associates<sup>6</sup> subjected allergic sera to electrophoresis and concluded from their experiments that the bulk of sensitizing antibody was not associated with gamma globulin. Menzel and associates,<sup>7</sup> using chemical and electrophoretic methods, studied the sera of ragweed-sensitive patients and found the skin-sensitizing antibody to be associated with the beta globulin and gamma globulin and possibly the  $\alpha_2$  fractions, but not with the  $\alpha_1$  or albumin fractions. Vaughan, Favour, and Jaffee<sup>8</sup> studied the skin-sensitizing antibody in ragweed hay fever patients, using Cohn's alcohol precipitation method (Method 10). The skin-sensitizing antibody was

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shown to be concentrated in the proteins of Fraction IIId, which was believed to consist primarily of beta globulins. Considerable activity was also noted in other fractions which contained almost no gamma globulin. In one serum containing reagins for ragweed pollen, Loveless and Cann<sup>9</sup> have found the activity to be distributed through the electrophoretic gamma and beta fractions. The study herein reported is an attempt to determine with which serum fraction the skin-sensitizing antibody is associated, using starch electrophoresis for serum fractionation.

#### METHOD

1. *Selection of Patients.*—Patients with hay fever or asthma, who demonstrated large positive reactions to various allergens by skin testing, were selected for this study. They were examined carefully to eliminate the possibility of previous infection with syphilis or virus hepatitis. A 30 ml. aliquot of fasting venous blood was taken under sterile conditions; the serum was withdrawn and stored at 2° C. until fractionation. A small quantity of the whole serum was passed through a Swinny-Seitz filter and a site was prepared in a nonallergic subject. Forty-eight hours later this site was tested with the appropriate antigen, and if a satisfactory passive transfer test was obtained the serum was fractionated.

2. *Methods of Separation.*—All sera were analyzed at 0.8° C. by free electrophoresis in Veronal buffer (pH 8.6, ionic strength 0.1<sup>16</sup>) in a Tiselius apparatus manufactured by Specialized Instruments Corporation, Belmont, California. Because of the higher optical sensitivity of this apparatus, the sera were diluted with buffer to 1 per cent protein concentration and dialyzed against the buffer for forty-eight hours prior to electrophoresis.

The relative concentrations of the various components were calculated from the schlieren patterns by the method of Tiselius and Kabat.<sup>10</sup> The total protein content of the sera was determined refractometrically and did not differ from normal values.<sup>16</sup>

For fractionation of sera into albumin and the various globulin fractions (alpha, beta, gamma), electrophoresis on supporting media was used. The findings of Kunkel and Slater,<sup>11, 12</sup> that potato starch molded into a rectangular block was an adequate medium for electrophoretic fractionation of complex protein mixtures, were confirmed and their method, slightly modified, was used throughout these experiments.

A trough of desired dimensions was made of a glass plate, and two 1.3 cm. Plexiglas bars were clamped to its sides. Thick wax paper was used for lining the trough. Filter paper pads were inserted at the ends of the trough and served also as bridges between the trough and buffer vessels. The potato starch\* was thoroughly washed twice with distilled water and excess moisture was removed on a Buchner funnel. Finally buffer solution was used as a displacing agent, providing an additional wash and resulting in the saturation of the starch with buffer ions. Sufficient buffer was then added to the starch to make it into a

\*Amend potato starch for chromatography was used in most of these experiments.

slurry. This was poured into the trough, excess moisture was removed by drying with filter papers, and finally the upper surface of the block was planed off with a wide spatula.

The serum to be separated was either injected lineally across the middle of the block or else mixed with some starch and poured into a rectangular hole cut out of the starch block. Good separation into straight and narrow bands was obtained when the width of the zone of application of the serum did not exceed 15 to 20 mm. and was uniform across the whole width of the starch block. This was best achieved with the second method of application. Application of the serum across the middle of the block prevented its displacement by chromatography and resulted in a sharpening of the zone of application during the two-hour equilibration with buffer which usually preceded electrophoresis. At the end of this period, the upper surface of the block was covered with a sheet of wax paper to minimize evaporation. The length of the block was 108 cm., its height was 1.3 cm., and the width varied from 5 to 10 cm., depending on the amount of serum applied. This varied between 2 and 6 ml.

The two buffer vessels filled to the same level were connected by filter paper wicks with the electrode vessels containing silver-silver chloride electrodes in 20 per cent potassium chloride solution. These electrodes were used in preference to the conventional platinum or carbon and steel electrodes, since no change in pH occurred. The buffer used was Veronal, at pH 8.6 and ionic strength 0.05. This ionic strength was used, rather than the usual ionic strength of 0.1, since it gave as good a resolution and allowed the application of higher voltages without undesirable increase in amperage. For good separation, the potential applied was about 1,000 volts and the current varied from 18 to 36 Ma., depending on the width of the starch block.

The electrophoretic separation was carried out in the cold room at  $2 \pm 1^\circ \text{C}$ . The temperature measured with a thermometer inserted into the block did not exceed  $14^\circ \text{C}$  and was usually around  $10^\circ \text{C}$ . The length of the experiments varied between eighteen and twenty-four hours, and the separation of the albumin peak from the gamma globulin peak ranged from 30 to 45 cm. The migration of the albumin could be seen easily, due to the color of the various pigments associated with it. At the end of the experiment, the region over which the serum fractions were distributed was determined by applying a filter paper strip along the surface of the block. After removal it was stained with bromophenol blue. This "print" revealed distinct bands corresponding to the various protein components.

For elution of the serum fractions from the starch, the block was cut into segments 1 cm. wide. These were placed in sintered glass funnels and eluted by displacement with saline under suction. This process was hastened with suction, and foaming invariably occurred. In the later experiments, a speedier and more convenient method of elution was used. This consisted of transferring the starch segments into plastic tubes provided with perforated bottoms which were covered by filters, 1 to 2 mm. thick, made of paper powder. The tubes were suspended in 40 ml. standard centrifuge tubes (about 2.5 cm. from the bottom

of the latter) on three "fingers" protruding from the sides to the center of the centrifuge tubes. Centrifugation at about 2,500 r.p.m. for three minutes was sufficient for elution. Addition of saline solution to each plastic tube, followed by further centrifugation, insured complete elution of the protein from each starch segment. This modification reduced the time of elution of 70 to 80 tubes to about three hours, eliminated foaming, and prevented any starch particles from being carried into the eluate. It was found that contamination of the eluate with small amounts of starch interfered with the determination of protein concentration, and such contamination was avoided by this method of elution. The total volume of the eluate of each segment amounted to about 7 to 9 ml. An equal aliquot of 1.0 ml. from each tube was used for protein determination by the method of Lowry and his associates,<sup>13</sup> and a plot of relative protein concentration led to a clear delineation of the zones containing the electrophoretically separable serum fractions. The various protein fractions were reconstituted by pooling the eluates as indicated by the graph and concentrating by dialysis against 25 to 35 per cent dextran\* solutions in standard Visking tubing. For comparison of the activity of the various fractions with that of untreated whole serum, all fractions were concentrated to the same volume as that originally used for electrophoretic separation. Lyophilization did not prove useful, as it resulted in concentrating, in addition to serum protein fractions, salts from the buffer and the saline to an undesirably high level. Concentration by dialysis against concentrated dextran solutions resulted in removal of both salts and water. To minimize the losses that occur in handling small volumes, concentration by dialysis was not carried beyond reducing the volume to about 5 or 6 ml. Final concentration to about 2 or 3 ml., when required, was achieved by partial lyophilization.

To determine whether the various chemicals used in the procedure caused skin irritation by themselves when injected into test subjects, two control fractions devoid of protein were prepared in each experiment. These were composed of the eluates from 10 to 15 cm. zones adjacent to the albumin and gamma globulin fractions and were concentrated by the same procedure.

In addition to characterizing the fractions from the curve obtained as described previously, each fraction was separately identified with its counterpart in the original serum by electrophoresis on filter paper. For this purpose the individual fractions were applied side by side with the untreated serum on a strip of filter paper (Whatman No. 1). After electrophoresis in a Veronal buffer of pH 8.6, ionic strength 0.1, in an apparatus similar to that described by Valmet and Svensson<sup>14</sup> and developed independently in this laboratory, the paper was dried and stained with amidosewarz. The staining method used was that described by Pucar<sup>15</sup> and was preferred to that with bromophenol blue, since it gave much deeper and more easily discernible color to the various protein bands. All fractions were passed through the Swinny-Seitz filter, especially adapted for handling small volumes, into sterile vials.

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\*Supplied by courtesy of Dextran Corporation, Yonkers, N. Y. This batch contained dextrans with a molecular weight distribution of around 58,000.

3. *Detection of Activity.*—The skin-sensitizing activity of the serum fractions obtained from starch electrophoresis was determined by passive transfer. The subjects used for testing were medical students who had no history of allergic disease. They were skin tested to the common allergens before receiving the serum fractions, and only those with negative reactions were used. The back of the recipient was washed with alcohol, and 0.1 ml. of the whole serum and each of the serum fractions were injected intracutaneously in duplicate. The prepared sites were marked with methylene blue. Forty-eight hours later, the back of the recipient was again washed with alcohol and the sites were tested. Volumes of 0.01 ml. of the antigen solutions were injected into each of the prepared sites. One set of the duplicate sites was tested with the antigen to which the patient was sensitive and the other set of duplicate sites was tested with an antigen to which the patient showed no reaction on intradermal skin testing. The antigens were also injected into nonprepared areas of the recipient's back and the results in the prepared sites were compared with these control tests. The results were read twenty minutes after injection of the antigen. The extent and depth of the wheal, pseudopod formation, and extent and depth of flare response were evaluated and the results, after comparison with the control tests, were graded 0 to ++++.

#### RESULTS

A series of tests was carried out on the sera of patients with marked sensitivity to various allergens, as determined by skin testing. Preliminary testing by passive transfer using whole serum showed that in some of the cases skin-sensitizing activity in the sera was not present or was very small. These sera were discarded and only those showing relatively large Prausnitz-Küstner tests were used. It also became apparent that the selection of cases should be based on the results of skin testing by the intracutaneous method, rather than by the scratch method. It was noted that in one case testing the skin of the patient by the scratch method gave a large positive wheal, while the intracutaneous test was negative. This was probably a quantitative phenomenon, since the antigen used for scratch testing was many times more potent than that used for intracutaneous testing.

After separation, the whole sera and fractions were tested by the Prausnitz-Küstner technique in the back of a non-allergic recipient. The first serum studied showed positive reactions for skin-sensitizing antibody to ragweed pollen and will be described later in this article. The next four sera showed negative reactions for the sera fractions. These sera were from persons who were not ragweed-sensitive but were sensitive to a variety of other allergens, such as cat dander, grass pollens, and some foods. There seemed to be several possible explanations of the inability to demonstrate skin-sensitizing antibody in the serum fractions of these patients.

The possibility that the negative reactions obtained might be due to individual variations in the recipients was eliminated by repeating the tests, using several medical students for the same serum and its fractions. The results were

TABLE I. SERUM PROTEIN ANALYSES IN RAGWEED-SENSITIVE PATIENTS (ELECTROPHORETIC DISTRIBUTION IN PER CENT)

| NAME                                 | AGE | SEX | DIAGNOSIS                          | ALBU-MIN | ALPHA <sub>1</sub> GLOBU-LIN | ALPHA <sub>2</sub> GLOBU-LIN | BETA GLOBU-LIN | GAMMA GLOBU-LIN | ALBUMIN GLOBU-LIN RATIO |
|--------------------------------------|-----|-----|------------------------------------|----------|------------------------------|------------------------------|----------------|-----------------|-------------------------|
| P. D.                                | 26  | F   | Extrinsic asthma-atopic dermatitis | 51.97    | 6.06                         | 11.08                        | 12.40          | 18.46           | 1.08                    |
|                                      |     |     |                                    | 53.10    | 6.29                         | 8.82                         | 13.15          | 18.63           | 1.13                    |
| D. B.                                | 29  | M   | Hay fever                          | 58.35    | 5.32                         | 10.81                        | 12.63          | 12.88           | 1.40                    |
| H. R.                                | 29  | M   | Hay fever                          | 56.55    | 5.68                         | 9.15                         | 14.71          | 13.90           | 1.30                    |
| G. L.                                | 30  | F   | Hay fever                          | 54.65    | 5.53                         | 7.24                         | 15.12          | 17.46           | 1.20                    |
| J. N.                                | 27  | M   | Hay fever                          | 51.97    | 4.49                         | 10.90                        | 16.41          | 16.23           | 1.08                    |
| D. C.                                | 36  | M   | Hay fever                          | 60.35    | 5.62                         | 9.23                         | 15.89          | 8.91            | 1.52                    |
| E. H.                                | 38  | F   | Hay fever                          | 58.73    | 5.56                         | 8.73                         | 17.68          | 9.29            | 1.42                    |
| R. J.                                | 27  | F   | Hay fever                          | 57.06    | 5.98                         | 7.82                         | 12.37          | 16.76           | 1.33                    |
| N. G.                                | 48  | M   | Hay fever                          | 59.39    | 6.17                         | 8.23                         | 14.93          | 11.28           | 1.46                    |
| G. C.                                | 49  | M   | Hay fever                          | 55.90    | 4.09                         | 8.78                         | 16.01          | 15.22           | 1.27                    |
| <i>Average</i>                       |     |     |                                    |          |                              |                              |                |                 |                         |
| Normal men (30 cases) age 22 to 35   |     |     |                                    | 59.64±   | 4.67±                        | 8.60±                        | 12.92±         | 14.33±          | 1.48±                   |
|                                      |     |     |                                    | 2.99     | 1.82                         | 1.43                         | 1.27           | 2.45            | 0.19                    |
| Normal women (14 cases) age 18 to 25 |     |     |                                    | 58.40±   | 5.17±                        | 9.81±                        | 12.42±         | 14.18±          | 1.40±                   |
|                                      |     |     |                                    | 2.31     | 0.73                         | 0.98                         | 1.18           | 1.76            | 0.14                    |

similar in all cases. It was known that serum stored at 2° C. retains its skin-sensitizing activity for many months, but the effect of aging upon serum fractions that are dissolved in buffer was not known. Subsequent tests with sera fractions which were positive showed that these fractions did lose activity, even when stored in the frozen state. In addition, the whole sera and sera fractions

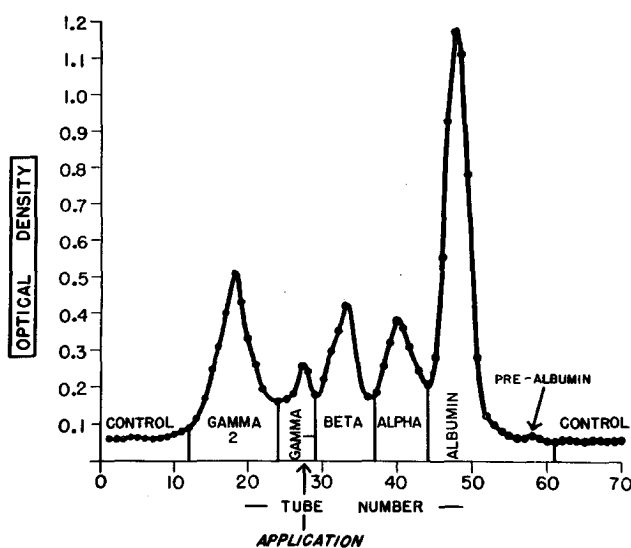


Fig. 1.—A protein distribution curve obtained by starch electrophoresis, showing the subdivision into various fractions.

were usually Seitz-filtered only once, but on occasion isolated fractions were filtered twice or more. It was found that the skin-sensitizing activity decreased markedly with each filtration. In view of these findings, it was decided to perform the fractionation procedure as rapidly as possible and to use the fractions

for Prausnitz-Küstner test as soon as they were obtained. Subsequent fractions were Seitz-filtered only once, immediately before they were used for passive transfer testing.

Since ragweed pollen appears to be one of the most antigenic of the common allergens and therefore might be expected to give rise to larger amounts of skin-sensitizing antibody in serum, it was decided that our attention would be directed, for the present, to sera of ragweed-sensitive patients.

The results of the free electrophoresis of sera from patients sensitive to ragweed pollen are given in Table I, together with the average values obtained in this laboratory for thirty men and fourteen women. A study of this table reveals a trend for the gamma globulin and/or beta globulin fractions in ragweed-sensitive sera to be somewhat elevated as compared with our normal values.

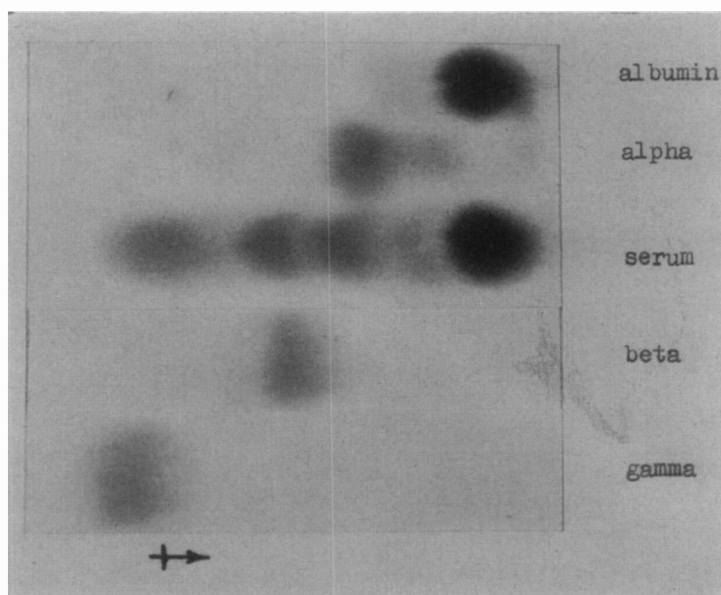


Fig. 2.—The characterization of the isolated serum fractions by paper electrophoresis.

A protein distribution curve obtained by starch electrophoresis and the subdivision into the various fractions is shown in Fig. 1. Due to a relatively large electro-osmotic effect, the gamma globulin fraction was found to move at pH 8.6 to the cathode. This confirms the findings of Kunkel and Slater.<sup>11, 12</sup> In the gamma globulin region, two peaks almost invariably were clearly delineated and are considered to be the two components, gamma<sub>1</sub> and gamma<sub>2</sub>. This split of the gamma globulin fractions usually is not detectable in free electrophoresis.

The alpha<sub>1</sub> globulin peak usually was not resolved and, as revealed by electrophoresis on paper, was included with the albumin peak (Fig. 2). In most sera a small peak, usually absent in free electrophoresis, preceded the albumin on the starch (Fig. 1).

TABLE II. PASSIVE TRANSFER REACTIONS IN RAGWEED-SENSITIVE PATIENTS

| NAME    | AGE<br>(YEARS) | SEX | DIAGNOSIS                              | INTRADERMAL<br>SKIN TEST TO<br>RAGWEED (1,000<br>PNU PER<br>MILLILITER) | AGE OF<br>SERUM AND<br>FRACTIONS<br>(DAYS) | PASSIVE TRANSFER TESTS TO RAGWEED<br>(1,000 PNU PER MILLILITER) |                         |                        |                       |                        |         |   |
|---------|----------------|-----|----------------------------------------|-------------------------------------------------------------------------|--------------------------------------------|-----------------------------------------------------------------|-------------------------|------------------------|-----------------------|------------------------|---------|---|
|         |                |     |                                        |                                                                         |                                            | WHOLE<br>SERUM                                                  | WHOLE<br>GLOBU-<br>LINS | GAMMA<br>GLOBU-<br>LIN | BETA<br>GLOBU-<br>LIN | ALPHA<br>GLOBU-<br>LIN | ALBUMIN |   |
|         |                |     |                                        |                                                                         |                                            |                                                                 |                         |                        |                       |                        |         |   |
| P. D.*  | 26             | F   | Extrinsic asthma-<br>atopic dermatitis | 8+                                                                      | 110                                        | ++++                                                            |                         | +++                    | +++                   | 0                      | 0       | 0 |
| P. D.** | 26             | F   |                                        |                                                                         | 34                                         | ++++                                                            | ++++                    | ++++                   | ++                    | 0                      | 0       | 0 |
| D. B.*  | 29             | M   | Hay fever                              | ++++                                                                    | 28                                         | ++++                                                            | ++++                    | ++                     | ++                    | 0                      | 0       | 0 |
| H. R.** | 29             | M   | Hay fever                              | 6+                                                                      | 28                                         | ++++                                                            | ++++                    | +++                    | ++                    | 0                      | 0       | 0 |
| G. L.** | 30             | F   | Hay fever                              | 5+                                                                      | 24                                         | ++++                                                            | ++++                    | ++++                   | ++                    | 0                      | 0       | 0 |
| J. N.** | 27             | M   | Hay fever                              | 8+                                                                      | 25                                         | ++++                                                            | ++++                    | +++                    | ++                    | 0                      | 0       | 0 |
| D. C.*  | 36             | M   | Hay fever                              | ++++                                                                    | 13                                         | +++                                                             |                         | +++                    | +++                   | 0                      | 0       | 0 |

\*Gamma fractions free of beta.

\*\*Gamma fractions containing some beta.

These sera and their fractions were tested for reaginic activity by passive transfer methods. Positive results were obtained in the fractions of seven sera from six ragweed-sensitive persons. Serum from one patient was obtained on two occasions with an interval of six months. Both sera and their fractions showed reproducible positive results. In four cases reaginic antibody was confirmed in the whole serum, but activity could not be demonstrated in the serum fractions. The seven positive results are shown in Table II. It can be seen that, in all cases, activity was demonstrable in the gamma and beta globulin fractions, but not in the alpha globulin and albumin fractions. In two cases, all the globulin fractions from the starch block were pooled together and, when tested, showed the same degree of whealing and erythema as the untreated whole sera. The control site prepared with fractions that did not contain protein gave negative reactions, as did all the sites tested with antigens to which the patients were not sensitive. While the techniques used in this study do not lend themselves to a quantitative interpretation of the data, it was noted that in four of the seven cases there was more activity in the gamma globulin than in the beta globulin fraction.

Subsequent to the Prausnitz-Küstner tests, these seven positive sera and their fractions were re-characterized as to their electrophoretic mobility and purity by paper electrophoresis (Fig. 2). The albumin and alpha globulin fractions were shown in all cases to be free of other serum protein components. The beta globulin fractions of the seven sera did not contain any detectable gamma globulin, but four of them contained traces of some faster-moving material with a mobility between that of alpha and beta globulins. The gamma globulin fractions of three of the seven sera did not reveal the presence of any other globulin fractions; the other four gamma globulin fractions showed traces of material moving with the mobility close to that of beta globulin.

It is obvious from these results that there was no skin-sensitizing activity associated with the albumin and alpha globulin fractions, that there was definitely activity in all beta globulin fractions, and that the activity in the slowest-moving component was almost certainly associated with gamma globulin.

#### DISCUSSION

The methods for serum fractionation used in previous studies of this nature have certain disadvantages. Separation by salting-out methods has been shown to result in fractions which contained more than one electrophoretically separable protein component.<sup>3, 17</sup> Alcohol fractionation methods suffer from the same disadvantage. The Tiselius electrophoresis method enables one to recover the fastest and slowest moving components in a relatively pure state. However, intermediate components are separable only with great difficulty and the amounts recoverable by this method are very small. The finding of previous workers using this method,<sup>3, 18</sup> that skin-sensitizing activity was associated with gamma globulin, is considered to be valid but conclusions regarding the activity of other fractions are open to question. The electrophoresis-convection method, although

a powerful tool for the separation of large quantities of material, produces fractions containing more than one electrophoretically separable component. Furthermore, it necessitates a relatively long period for the complete processing of serum.

Starch electrophoresis yields reproducible fractionations when used on the same serum, and the relative protein concentrations of the various fractions are of the same order of magnitude as those calculated from the schlieren patterns of free electrophoresis. The advantages of starch electrophoresis methods are rapidity of the separation, simultaneous separation of the sera into their electrophoretically separable components, and the possibility of direct characterization of the various components. Furthermore, in common with all electrophoretic techniques, it is one of the mildest methods of serum fractionation.

The results presented in Table I do not allow any conclusive generalization regarding the protein distribution in sera of ragweed-sensitive patients except that the albumin-globulin ratio shows a trend toward lower values than those of normal persons. Similar results in infective asthma were reported by Cooke and his co-workers.<sup>6</sup>

The passive transfer experiments demonstrate that skin-sensitizing activity in the sera of ragweed-sensitive patients is associated with beta and gamma globulin fractions and is not associated with albumin and alpha globulin fractions. The results of the characterization of the individual fractions by paper electrophoresis show conclusively that the albumin and alpha globulin components do not contain any other slower-moving components. Some of the beta globulin fractions contain traces of a component moving with a mobility close to that of  $\alpha_2$  globulin, but do not contain any slower-moving components. Thus, the activity found in the beta globulin fraction cannot be due to any other serum protein component. Three of the gamma globulin fractions showed no trace of other serum proteins, and the skin-sensitizing activity of these fractions cannot be attributed to faster-moving components. In the other four cases, traces of material with a mobility approaching that of beta globulin were found in the gamma globulin fraction. However, the high degree of activity found in these fractions, together with the unequivocal findings in the aforementioned three instances, is considered to be strong evidence that the skin-sensitizing activity in these fractions was in fact associated with gamma globulin. Further experiments, designed to eliminate interzonal fractions, are in progress and it is hoped that they will clarify this matter further. Since this work was completed, further studies have indicated that activity probably resides in the gamma<sub>1</sub> fraction primarily. This would explain the finding of activity in both beta and gamma fractions in the present work. In four cases, skin-sensitizing activity, while demonstrable in the whole sera of ragweed-sensitive patients, could not be elicited in the serum fractions obtained by starch fractionation. It is not apparent from our data why this activity could not be demonstrated after fractionation.

## SUMMARY

1. The separation of ten sera from ragweed-sensitive patients into their electrophoretically separable protein components, using starch electrophoresis, is described. The advantages of the method are discussed and several modifications are presented.

2. The resulting sera fractions were tested by passive transfer techniques to determine the localization of skin-sensitizing antibody present in the whole sera.

3. It was found that the albumin and alpha globulin fractions have no skin-sensitizing activity, while both beta and gamma globulin fractions demonstrate significant skin-sensitizing activity. These findings and the results of previous work of this nature have been discussed.

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