# Immunoelectrophoresis of Serum in Progressive Systemic Sclerosis (Diffuse Scleroderma).* (26551)

AVINOAM ZLOTNICK† AND GERALD P. RODNAN (Introduced by Jessica H. Lewis)
*Depts. of Pathology and Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa.*

Since immunoelectrophoresis was introduced by Grabar and Williams(1) about 30 distinct proteins have been recognized in normal human serum(2), and as Grabar predicts many more will be described(3). These proteins appear in immunoelectrophoresis as specific precipitation arcs which can be identified by their characteristic mobility or configuration, by special staining procedures, or by absorbing the immune antiserum with certain purified proteins. The present study is concerned with paper strip and immunoelectrophoresis of the serum of patients with progressive systemic sclerosis (P.S.S.), in order to further characterise the protein abnormalities in this disorder(4,5).

*Materials and methods.* The serum samples used in this study were obtained from 15 healthy individuals and 15 patients with well-documented progressive systemic sclerosis in whom the diagnosis of scleroderma was confirmed by skin biopsy. Serum was separated from venous blood and kept frozen from several days to 2 months prior to examination. Immunoelectrophoresis was performed with Scheidegger's micro apparatus, using 25 × 75 mm microscopic slides(6). A 2% agar solution prepared in sodium barbital buffer, pH 8.2, ionic strength 0.02, was used as a medium for electrophoretic separation. Two parallel wells were cut out in the agar plate; one was filled with the pathological serum and the other with a normal serum. After 35 minutes of electrophoretic separation under a potential of 6 V/cm, a central trough was cut out and the immune antiserum was introduced. Diffusion was allowed to take place for 24 hours and the slides then washed in buffered saline for 24-48 hours. After drying, the slides were stained with azocarmin G.

All the immunoelectrophoretic studies were performed with antisera #511 and #512 from horses immunized with whole normal human serum, obtained from the Pasteur Institute in Paris. On several occasions, however, rabbit anti-alpha$_2$ macroglobulin serum and rabbit anti-normal human serum, obtained from Behringwerke in Marburg, were also used.‡ The results presented in Table I are those obtained using undiluted sera and antisera. An attempt to obtain clearer patterns by diluting either the serum or antiserum usually failed to improve the sharpness of the arcs.

Paper electrophoresis was performed with a Spinco Model R paper electrophoresis apparatus, and evaluation of the stained paper strips stained by alcoholic bromphenol blue was done by a Spinco Analytrol instrument. Total serum protein was determined by a modified biuret method(7). The results are given in Table I.

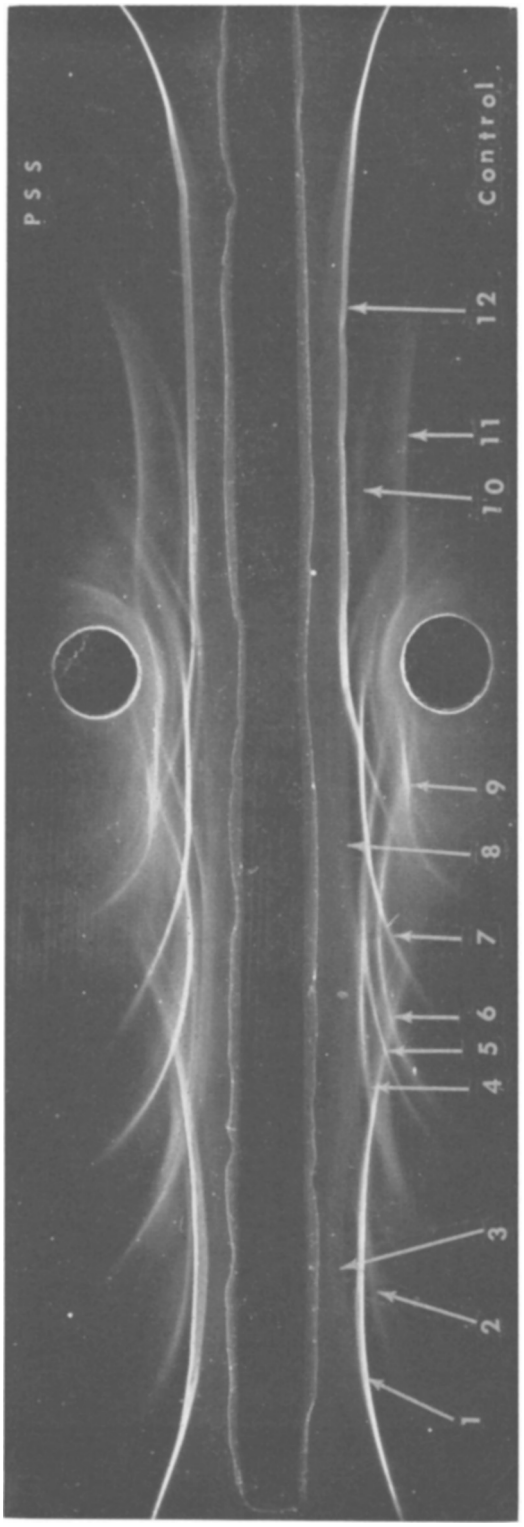
*Results.* The different immunoelectrophoretic precipitation lines obtained with antiserum #511 are illustrated in Fig. 1. The upper pattern is of a patient with progressive systemic sclerosis and the lower is of a normal control.

The following precipitation lines were found both in the normal controls and in the patients under study. *Albumin*: Two prealbumins, rho$_1$ and rho$_2$, were observed together only once. In most cases they were either absent or only one of them was present. (They were absent in the sera illustrated in Fig. 1.) The serum albumin formed a prominent arc which extended to the region of the alpha$_2$ globulin. *Alpha globulin*: The alpha$_0$ globulin appeared as a hazy complex inside the curvature of the albumin arc. This com-

* Supported by grants from U.S.P.H.S. and Health Research and Services Foundation, Pittsburgh. Publication #276 of Dept. of Pathology, Univ. of Pittsburgh School of Med., Pittsburgh, Pa.

† On leave of absence from Department of Medicine A, Hadassah Univ. Hospital and Med. School, Jerusalem, Israel.

‡ We wish to thank Prof. H. E. Schultze for kindly supplying us with a sample of rabbit anti-alpha$_2$ macroglobulin serum.




plex has been referred to as the × fraction and is identical with orosomucoid(3). The alpha$_1$ globulin appeared as a large, broad line which was accentuated at its cathodic extremity. According to Burtin(3), this line does not show important variation even in pathological sera. Our findings concurred. In the alpha$_2$ region, 3 components were found. The arc nearest to the antibody trough was identified with haptoglobin, while the outer arc showed a slower mobility and was identified as alpha$_2$ macroglobulin. Between these precipitation lines, a third line was sometimes present. This is probably the ceruloplasmin. Because this line was not always present even in the controls, it does not figure in Table I. *Beta globulin*: With antiserum #511, several beta$_1$ lines were found. Of these we shall mention the beta$_1$ siderophilin, an unidentified beta$_1$ globulin, and the beta$_1$ lipoprotein complex which originates from the antigen well and is the farthest from the antibody trough. This latter gives a positive staining with Sudan black. In the beta$_2$ region, the beta$_2$M and the beta$_2$A globulins run parallel to the gamma globulin. The beta$_2$A was sometimes missing in the controls as well as in the pathologic sera, and therefore does not figure in the Table. *Gamma globulin*: The gamma globulin fraction usually presented a long, asymmetric single line which extended up to the alpha$_2$ region. In 4 normal sera, however, there was a splitting at the anodic extremity.

The results of immunoelectrophoretic examination of the sera from 15 patients with progressive systemic sclerosis, as compared to normal controls, are given in Table I. Pertinent changes were observed in alpha$_0$, alpha$_2$ and gamma fractions. The alpha$_0$ was increased in 7 out of 15 cases. This manifested itself by a thick and clear precipitation line. The alpha$_2$ macroglobulin was increased

FIG. 1. Immunoelectrophoretic patterns of a patient (No. 6) with progressive systemic sclerosis (PSS) and a normal control. 1. Albumin. 2. Alpha$_0$ globulin. 3. Alpha$_1$ globulin. 4. Alpha$_2$ haptoglobin. 5. Ceruloplasmin (2). 6. Alpha$_2$ macroglobulin. 7. Siderophilin. 8. Beta$_1$ globulin. 9. Beta$_1$ lipoprotein. 10. Beta$_2$A globulin. 11. Beta$_2$M globulin. 12. Gamma globulin. Note splitting of the gamma globulin in the pathological serum.

TABLE I. Immuno- and Paper Strip Electrophoresis of Serum Proteins in Progressive Systemic Sclerosis.

| Patient | Age, race, sex | Immuno-electrophoresis | | | | | | Paper strip electrophoresis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\alpha_0$ | $\alpha_2$ haptogl. | $\alpha_2$ macrogl. | $\beta_2$M globulin | $\gamma$ globulin | Total protein | Albumin† | $\alpha$-1 | $\alpha$-2 | $\beta$ | $\gamma$ |
| 1 | 37W ♀ | + | + | + | ↑ | >— | 6.63 | 3.96 | .27 | .51 | .71 | 1.18 |
| 2 | 42W ♂ | + | ↑ | ↑ | + | + | 6.95 | 3.68 | .36 | .76 | .93 | 1.21 |
| 3 | 52W ♀ | ↑ | + | ↑ | + | >— | 7.54 | 3.79 | .32 | .79 | .83 | 1.82 |
| 4 | 38W ♀ | + | + | ↑ | + | >— | 7.81 | 4.74 | .40 | .69 | .75 | 1.23 |
| 5 | 70W ♀ | ↑ | ↑ | ↑ | + | + | 6.96 | 3.77 | .23 | .60 | .97 | 1.40 |
| 6 | 26W ♀ | ↑ | + | ↑ | ↑ | >— | 6.27 | 3.33 | .28 | .84 | .77 | 1.05 |
| 7 | 15W ♀ | + | + | ↑ | + | + | 6.83 | 3.80 | .29 | .76 | .70 | 1.27 |
| 8 | 37W ♀ | ↑ | ↑ | ↑ | ↑ | + | 7.05 | 4.59 | .10 | .34 | .63 | 1.40 |
| 9 | 35W ♀ | ↑ | + | + | ↑ | >— | 8.83 | 3.58 | .35 | .50 | .86 | 3.54 |
| 10 | 44W ♀ | + | + | ↑ | ↑ | >— | 6.58 | 3.47 | .25 | .72 | .76 | 1.38 |
| 11 | 68W ♂ | + | + | + | + | + | 6.46 | 3.07 | .29 | .87 | .65 | 1.58 |
| 12 | 58W ♂ | ↑ | + | + | + | + | 7.86 | 4.36 | .31 | .68 | 1.05 | 1.48 |
| 13 | 36W ♂ | + | + | + | ↑ | >— | 6.81 | 2.83 | .39 | .76 | .95 | 1.87 |
| 14* | 35W ♀ | ↑ | ↑ | ↑ | + | + | 7.52 | 3.44 | .29 | .54 | .80 | 2.46 |
| 15 | 47W ♀ | + | + | + | + | >— | 7.85 | 3.52 | .27 | .99 | 1.06 | 2.01 |
| | | | | | Normal range | | 6.15–8.11 | 3.81–5.61 | .19–.35 | .31–.75 | .42–.90 | .52–1.40 |
| | | | | | S.D. | | .49 | .45 | .04 | .11 | .12 | .22 |

* Sjögren's syndrome and P.S.S.

† All values in g %.

+, Normal length, thickness, and configuration of precipitin arc.
↑, Increase in thickness and sometimes length of precipitin arc.
>—, Splitting of precipitin arc.

in 9 cases; beta$_2$M globulin was increased in only 6 cases. Quantitative changes could not be observed in the gamma globulin fraction; however, a splitting of the anodic extremity of the precipitation line was a notable finding in 8 cases. No deviations from the normal were noted in serum albumin, alpha$_1$ globulin, beta$_1$ siderophilin, and beta$_1$ globulin.

Table I also provides the results of paper strip electrophoretic examination of the sera from these patients. There was a slight increase in alpha$_1$ globulins in 2 patients and in the alpha$_2$ globulins in 4. A significant increase in gamma globulins was observed in 8 patients, ranging from 1.48 g % to 3.45 g %. No strict relationship could be found between the variation in the composition of the serum proteins revealed by immunoelectrophoresis and paper strip electrophoresis.

*Discussion.* Our results indicate that immunoelectrophoresis complements paper strip electrophoresis in revealing delicate variations not detected by the latter. Analysis of the different precipitation lines is not always easy, especially where quantitative aspects are concerned. According to Burtin(3), an increase of a certain protein fraction in a complex protein mixture, such as serum, manifests itself in the immuno-electrophoretic diagram by an increase in thickness or length or clearness of the particular precipitation line. Sometimes this line may split in its center into several components which remain united at their extremities. Being aware of the limits of this method of quantitation, we listed a protein as increased only when the corresponding precipitation line was markedly thicker and sometimes also longer than the corresponding line of the control serum.

We could thus observe an increase in the alpha$_0$ globulin and in the alpha$_2$ macroglobulin fractions which was not evident in paper strip electrophoresis. The increase in the alpha$_0$ fraction, probably identical with the seromucoid or orosomucoid, is of particular interest, because the orosomucoids have been reported as being elevated in the active stage of other systemic rheumatic disorders(8). The importance of the increase in the alpha$_2$ macroglobulin is not clear. This line has been found to be increased also in rheumatoid arthritis(9).

The splitting of the gamma globulin arc at its anodic extremity is also of particular

interest. Edelman *et al.*(10) in an extensive immunological study of human gamma globulin reported that different purified human gamma globulin fractions give 2 precipitation lines with sera of rabbits immunized against certain pathological human sera. Similar results were reported by Burtin with special horse anti-human sera(3). This indicates that normal human gamma globulin has at least 2 antigenic determinants. The more common splitting of the gamma globulin in progressive systemic sclerosis, as compared to the control group, suggests that the morbid process may lead to an increase in the lesser of these antigenic determinants. Similar splitting of the anodic extremity of the gamma globulin has been described in hepatic cirrhosis(11).

Immunoelectrophoretic studies of sera from 8 patients with rheumatoid arthritis were reported by Cleve and Hartmann(12) who found an increase in the $alpha_1$ globulin, $alpha_2$ haptoglobulin, and in the $beta_2$M fraction. Similar observations were reported by Seligman(3) in systemic lupus erythematosus. Here however, the outstanding feature was a diminution or complete absence of the $beta_1$A fraction. The findings in P.S.S. differ from those in the above cited studies in that a significant increase in $alpha_2$ macroglobulin fraction was found. This and the less frequent increase in the $beta_2$M fraction may be related to the use of different antisera.

Immunoelectrophoresis adds some interesting data to the variations in composition of the serum proteins in progressive systemic sclerosis. It does not, however, give a particular picture characteristic of this disease to warrant its routine use for diagnostic purposes. The increase in the $alpha_2$ macroglobulin in this disease and in rheumatoid arthritis may point to a relationship worthy of further investigation.

*Summary.* Immuno- and paper strip electrophoresis were performed on the sera of 15 healthy individuals and 15 patients with progressive systemic sclerosis. An increase was observed in the $alpha_1$ globulin in 7 patients, in the $alpha_2$M globulin in 9, and in $beta_2$M globulin in 6. Splitting of the anodic extremity of the gamma globulin was more common in the pathological sera than in the controls. No relationship was found between these changes and those observed with paper strip electrophoresis.

We wish to express our appreciation to Dr. W. O. Weigle for his helpful advice and criticism.

1. Grabar, P., Williams, C. A., *Biochem. Biophys. Acta,* 1953, v10, 193.

2. Heremans, J. F., *Clin. Chim. Acta,* 1959, v4, 639.

3. Grabar, P., Burtin, P., *Analyse Immuno-électrophorétique.* Paris: Masson et Cie, 1960.

4. Ogryzlo, M. A., Maclachlan, M., Dauphinee, J. A., Fletcher, A. A., *Am. J. Med.,* 1959, v27, 596.

5. Rodnan, G. P., Etiology and pathogenesis of progressive systemic sclerosis and dermatomyositis. In *Hahnemann Symposium on Inflammation and Diseases of Connective Tissue,* to be published by W. B. Saunders Co., Philadelphia. in press.

6. Scheidegger, J. J., *Internat. Arch. Allergy,* 1955, v7, 103.

7. Gornall, A. G., Bardawill, C. J., David, M. M., *J. Biol. Chem.,* 1949, v177, 751.

8. Decker, B., McGuckin, W. F., McKenzie, B. F., Slocumb, C. H., *Arth. and Rheumat.,* 1960, v3, 49.

9. Schultze, H. E., Personal Communication.

10. Edelman, G. M., Heremans, J. F., Heremans, M. T., Kunkel, H. G., *J. Exp. Med.,* 1960, v112, 203.

11. Scheidegger, J. J., Zahnd, G., *Helvet. Med. Acta,* 1957, v24, 499.

12. Cleve, H., Hartmann, F., *Klin. Wschr.,* 1957, v35, 334.

Received Februarv 15. 1961. P.S.E.B.M., 1961, v107.