
DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS
BY ELECTROPHORESIS

BY

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Immunological and other laboratory indices can reflect various aspects of disturbed metabolism in patients. It is natural that all the links in this complex chain are not disturbed simultaneously or to the same degree. As protein metabolism is the most essential and universal, the most typical disturbances observed in collagen diseases can be revealed by the study of this process.

Nesterov and Sachkov (1961a, b), and Sachkov (1962), studying the antigenic properties of serum protein in patients with rheumatic fever and other collagen diseases, have demonstrated an antigen common to the entire group of collagen diseases, and antigens specific for rheumatic fever, rheumatoid arthritis, and systemic lupus erythematosus. This paper describes the first attempts to evaluate the clinical significance of the antigen specific for systemic lupus erythematosus.

Methods

The experimental techniques used in this study were as follows:

(1) Ten patients were selected in whom the diagnosis of acute and subacute systemic lupus erythematosus had been definitely established clinically and by the presence of L.E.-cells and antibodies to desoxyribose-nucleic acid (DNA). Serum from these patients was fractionated electrophoretically on starch by the method of Grabar (1954, 1957) and Grabar and Williams (1955).

(2) Electrophoretically homogenous γ globulins obtained from these patients were used to immunize and re-immunize rabbits by various techniques. Antiserum No. 6006, which was used in the experiments reported in this paper, was produced by the following process:

1 ml. of a 1·5 per cent. solution of the human γ globulin was injected intravenously into the rabbits daily for 3 days. On the 4th day, 1·5 ml. was injected intraperitoneally. After 12 days, the rabbits were bled (20-30 ml.) and the rabbit serum was tested against patients’ serum by the method described below under (3). After 3 to 4 months, the rabbits were re-immunized; for this 1·5 ml. of the original 1·5 per cent. solution of human γ globulin was injected intraperitoneally on the first day and the same amount intravenously on the second day. The animals were bled on the 7th day after re-immunization. If a satisfactory antiserum was not obtained after two or three re-immunizations, that animal was discarded. Usually only one out of twelve to fifteen immunized rabbits produced a satisfactory antiserum.

(3) In developing modifications of the method of electrophoresis, we made use of the published findings of Grassman and Hübner (1953), Marechal (1955), and Bustamante (1957).

Using a special applicator, 0·005 ml. of the patient's serum to be investigated was placed on a strip of filter paper (No. 2043, Schleicher and Schüll) previously moistened with buffer (LKB 3276 GB 10) which gives good separation, and a similar quantity of rabbit antisera was applied to the same place. Nine strips of paper measuring 400 × 40 mm. were run simultaneously in the electrophoresis apparatus. On completion of electrophoresis the strips were fixed, stained with amido black, washed, dried, and rendered translucent with colourless acryl-pistachio varnish. After densitometry the sizes of the various protein fractions, expressed as a percentage of the total protein, were determined with a planimeter. In the same experiment, electrophoresis was also carried out on 0·01 ml. of the patient's serum and 0·01 ml. of the rabbit antiserum on separate strips.

(4) A positive antigen-antibody reaction was considered to be present if the percentile content of the γ globulin in the mixed sera exceeded the mean of the percentile content of γ globulin of the human and rabbit sera run separately. To reduce experimental error, two or three electrophoretograms were prepared from each serum and from the mixture, and the mean values were used to calculate the result.

(5) L.E.-cells were determined by the method of Snapper and Nathan (1955), and serum antibodies to
DNA were determined by the modified Ouchterlony methods of Hartmann (1957), Hartmann and Toilliez (1957), and Deicher, Holman, and Kunkel (1959).

Experimental Studies

Reactions with Antiserum No. 6006 were performed with the sera of eighty patients with different diseases; 44 with systemic lupus erythematosus, twelve with systemic scleroderma, four with rheumatoid arthritis, ten with rheumatic fever, and ten with different non-collagen diseases.

Antiserum No. 6006 to the gamma-globulin of patients with lupus erythematosus gave a positive result with the sera of two of the twelve patients with scleroderma (mean reaction $-1.0 \pm 0.9$ per cent.), of one female patient of the four with rheumatoid arthritis (mean reaction $+0.8 \pm 0.4$ per cent.), of one female patient of the ten with rheumatic fever (mean reaction $-0.9 \pm 0.4$ per cent.), and of one male patient of the ten with various non-collagen diseases (mean reaction $-1.1 \pm 0.1$ per cent.).

Positive results were regularly found only in the 44 patients with systemic lupus erythematosus, who formed the largest group tested. They included two males and 42 females and their ages ranged from 18 to 55 years.

Lesions were found in various organs and bodily systems of all these 44 patients (Table). Joint changes in the form of arthralgia and arthritis (often rheumatoid-like) were the lesions most frequently seen (in forty patients). Skin eruptions, ranging from the classical "butterfly" to small atypical discoid plaques on the face and ears, or over the involved joints, were found in 35 patients. There were also cardiac lesions in 29 patients, pulmonary lesions in seventeen, active polyserositis or scars of previous serositis in twenty, renal lesions (in the form of diffuse or focal nephritis) in sixteen, and neurological lesions (ranging from slight polymyelitis to transverse myelitis and encephalopathy) in twelve; abnormalities of the reticulo-endothelial systems were also frequently noted. Almost half the patients had the triad of rash, arthritis, and polyserositis which is characteristic of lupus erythematosus.

Hypergammaglobulinaemia was present in forty patients, a persistent increase in the erythrocyte sedimentation rate (E.S.R.) to between 20 to 70 mm./1st hr in 38, and leucopenia in 23. The diagnosis was confirmed in 27 patients by the isolation of L.E.-cells, and in nine at autopsy.

Thus only cases with an indubitable diagnosis of systemic lupus erythematosus were included in the series studied. Taking into consideration the acuteness of the disease process, the clinical symptoms, and the duration of remissions, the 44 patients were divided into three groups according to the course of the disease: 28 acute, eight subacute, and eight chronic (Fig. 1, opposite).

Classical Characteristics of 44 Patients with Lupus Erythematosus According to the Course of the Disease

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Course of Disease</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1) Acute</td>
<td>(2) Subacute</td>
</tr>
<tr>
<td>Articular Lesions</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>Skin Lesions</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Cardiac Lesions</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary Lesions</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Polyserositis</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Renal Lesions</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Neurological Lesions</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Hepato-lienal Lesions</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Total Patients</td>
<td>28</td>
<td>8</td>
</tr>
</tbody>
</table>

(1) Maximal Reactions (+8·4; +8·8; +8·8 per cent. Average 8·7 $\pm$ 0·09) were found in three patients with pronounced disease activity indicated by high temperature, pain, joint effusion, and damage to certain internal organs. None of these
patients had received steroid therapy before admission to the clinic. The following case is an example:

**Case 1, a woman aged 26 years**, fell ill in 1957 after childbirth, with pains in the legs, general weakness, and an E.S.R. of 40 mm./1st hr. At the beginning of 1960 she developed a high fever with arthritis; in September, 1960, she had cardiac pain with electrocardiogram changes, and later showed urinary abnormalities and an E.S.R. in the range of 50 mm./1st hr.

On admission to the clinic she complained of joint and cardiac pains, shortness of breath, and general weakness. Objectively her general condition was satisfactory, but she was feverish (38° C.). Many joints were painful on movement and the right ankle was swollen. All groups of lymph nodes were palpable. A systolic murmur was heard over the apex and pulmonary artery, but the heart contours were unchanged and the remaining organs were normal.

**Blood Analysis**: Hb 62 per cent.; white blood cells 4,000; E.S.R. 66 mm./1st hr; total serum protein 8·06 per cent. (albumin 30·8 per cent., α 2 9·1 per cent., γ 2 15·8 per cent., β 2 28·8 per cent.). Formol test 4+; D.F.A. 0·310; sialic acid 0·330. L.E.-cells 40 : 1,000. Waaler-Rose test negative. Reaction with Antiserum No. 6006 +8·8 per cent.

**Urine Analysis**: Protein 1·65 per cent.; specific gravity 1011; leucocytes 30 to 40 per field; lixival erythrocytes 3 to 4 per field.

**Moderate Reactions**.—Signs of disease exacerbation were found in thirteen patients with reactions ranging from +1 to +6·7 per cent. (average 3·3 ± 0·2). In these patients the exacerbation developed during prolonged therapy with supportive doses of hormones and was characterized by various clinical manifestations, including active L.E. nephritis without tendency to renal failure in nine patients (prolonged observation showed that only one female patient died from renal failure). The following case is an example:

**Case 2, a woman aged 19 years**, was admitted to the clinic with systemic lupus erythematosus in exacerbation during the sixth year of disease. She had bilateral interstitial pneumonia with high fever, abundant wet rattle in the lungs due to L.E. pneumonitis with pulmonary hypertension and early phenomena of pulmonary heart, extensive exudative erythema on the face and extremities, persistent polyarthritis with myalgia and
fibrositis, diffuse nephritis, and hepato-splenic syndrome.

**Blood Analysis:** Hb 56 per cent.; white blood cells 5,100; E.S.R. 68 mm./1st hr; total serum protein 9:35 per cent. (albumin 29:7 per cent., \(\alpha_2\) 3:9 per cent., \(\alpha_1\) 16:3 per cent., \(\beta\) 9:6 per cent., \(\gamma\) 40:6 per cent.). Formol test 4+; D.F.A. 0:334; sialic acid 0:200; C-reactive protein 3+. L.E.-cells numerous. Reaction with Antiserum No. 6006 +4-2 per cent.

**Urine Analysis:** Protein 3:3 per cent. (more often 0:99 per cent.); specific gravity 1010-1024; changed and unchanged erythrocytes 12 to 15 per field with hyaline, granular, and waxy casts.

(iii) Borderline Reactions.—In five patients the reactions ranged from -1 to +0:9 per cent. (average +0:15 ± 0:27); all of them had been admitted to the clinic with persistent arthralgia and lymphadenopathy and had already received a significant amount of steroid hormone therapy. In four patients the liver and spleen were enlarged. In four patients renal changes were not found, but one female patient had renal disease and her reaction to Antiserum No. 6006 was -1 per cent., i.e. at the lower limit of normal. The following case is a typical example:

**Case 3, a woman aged 18 years,** had been ill for 1 year with recurrent arthritis, fever, and asthenia; 2 months before admission she had had a severe exacerbation during which polyserositis developed, followed by vasculitis of the central nervous system and involvement of the gastro-intestinal tract. She was given 30 mg. prednisolone daily with good effect. The temperature fell to normal, and the general condition was improved, except for arthralgia, cardiac pain, and tachycardia.

**Blood Analysis:** Hb 48 per cent.; E.S.R. 62 mm./1st hr; total serum protein 6:55 per cent. (albumin 29:3 per cent., \(\alpha_2\) 9:9 per cent., \(\alpha_1\) 15:8 per cent., \(\beta\) 14:6 per cent., \(\gamma\) 30:5 per cent.). D.F.A. 0:262; sialic acid 0:261. L.E.-cells 10 : 1,000. Reaction with Antiserum No. 6006 +0:8 per cent.

(iv) Strongly Negative Reactions.—In seven patients the reactions ranged from -2:2 to -6:6 per cent. All had changes in the joints, heart, and lungs; four patients had pronounced renal disease with a tendency to renal failure, and two of these soon died of uremia. The following case is a typical example:

**Case 4, a woman aged 37 years,** fell ill in 1956 with dermatitis considered to be photodermatosis. In March, 1959, during pregnancy, the dermatitis became more severe with oedema and changes in the urine. After childbirth the dermatitis became erosive, with temperature 39° C. and polyarthritis. Severe diffuse nephritis developed with marked oedema, blood pressure 170/110, protein in urine 9:9 to 16 per cent., hypoproteinaemia 4:2 per cent., and hypercholesterolaemia. She became anaemic (Hb 42 per cent.), and the E.S.R. was 58 mm./1st hr, with numerous L.E.-cells. Treatment gave temporary remission, but in March, 1960, she had a new exacerbation, with rash on the face and chest, general weakness, increased oedema, headache, dyspepsia, and oliguria. She was again admitted to the clinic with exacerbation of L.E. nephritis (anasarca, ascites, blood pressure 150/90, urinary output 400 ml./24 hrs) and marked dyspepsia.

**Blood Analysis:** Hb 46 per cent.; white blood cells 4,600; E.S.R. 58 mm./1st hr; total serum protein 5:25 per cent. (albumin 24:7 per cent., \(\alpha_2\) 9:2 per cent., \(\alpha_1\) 13:1 per cent., \(\beta\) 15 per cent., \(\gamma\) 37:8 per cent.); cholesterol 220 mg. per cent., retained nitrogen 75 mg. per cent. C-reactive protein 2+. L.E.-cells numerous. Reaction with Antiserum No. 6006 -5:4 per cent.

**Urine Analysis:** Protein 19 per cent.; specific gravity 1019; changed erythrocytes 10 to 12 per field, with hyaline and granular casts.

Despite treatment with large doses of steroid hormones, her condition remained poor, ureaemia increased (retained nitrogen 119, 155, and 189 mg. per cent.), urinary protein 33 to 66 per cent., specific gravity 1016. The reaction with Antiserum No. 6006 became -8 per cent. and the patient died with symptoms of increased ureaemia. Autopsy confirmed the diagnosis of systemic lupus erythematosus with L.E. nephritis.

**GROUP 2—SUBACUTE COURSE (8 CASES)**

All these patients had what was mainly a dermo-articular syndrome without fever. The general health was not greatly disturbed. There were only moderate (dystrophic) heart changes in three patients, and a transient hepato-splenic syndrome was observed in two. The erythrocyte sedimentation rate was not more than 40 mm./1st hr (usually about 20 mm./1st hr). Four patients also had moderate leucopenia (from 3,000 to 4,400) and very small \(\gamma\) globulin changes (from 24-2 to 29 per cent., but in one patient 40-1 per cent.).

L.E.-cells were found in only four patients: one had 1 or 2 L.E.-cells per 1,000 leucocytes, two had 1 or 2 L.E.-cells per preparation, and the fourth had only "rosettes".

The reaction with Antiserum No. 6006 was positive (ranging from +0-2 to +9-3 per cent.) in seven female patients, but in the eighth patient the reaction was negative (-2-3 per cent.) and in this
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patient the authors failed to elicit clinical or laboratory evidence of active lupus erythematosus.

In this group of patients, as in those in which the disease took an acute course, the same association was noted. Earlier and more active cases with significant effusions gave a rather pronounced reaction (up to +9·3 per cent.), but the average was lower (+3·6 ± 0·9 per cent.). The following is a typical case:

Case 5, a woman aged 23 years, fell ill with lupus erythematosus in January, 1959. Deterioration developed in the summer of 1960, and she was treated with chloroquine with good effect, but an exacerbation began in March, 1961. She then had "butterfly" rash on the face, enlarged tender lymph nodes in the left side of the neck (lymphadenitis), and slight systolic murmur at the cardiac apex with accentuation of P.2.

Blood Analysis: Hb 66 per cent.; white blood cells 4,700; E.S.R. 34 mm./lst hr; total protein 8·7 per cent. (albumin 43·8 per cent., $\alpha_1$ 5·9 per cent., $\alpha_2$ 13·4 per cent., $\beta$ 15·3 per cent., $\gamma$ 21·5 per cent.). Fibrinogen 0·61 per cent.; D.F.A. 0·300; sialic acid 0·162. L.E.-cells not found. Reaction with Antiserum No. 6006 +5·8 per cent.

GROUP 3—CHRONIC COURSE (8 cases)

The duration of disease in these eight patients was 10 to 29 years, and they had had remissions lasting for 2, 3, 5, or 10 years, during which they were able to work. However, five showed signs of transition to an acute course with characteristic multiple symptoms. In addition to the dermo-articular syndrome, cardiac lesions were always found, and six had polyserositis with minimal effusion and a tendency to the rapid appearance of scars. L.E. pneumonitis, a chronic process in the interstitial tissue of the lungs, with a discoid atelectasis, elevation of the diaphragm, and significant disturbance of respiratory function was found in three patients. Four patients had renal changes of focal nephritis with moderate albuminuria, without disturbance of lipid or protein metabolism, but with oedema and hypertension.

The E.S.R. was usually much elevated (40 to 60 mm./lst hr), as in those with the acute form of the disease. Only three patients had slight leucopenia (range 4,000 to 4,800). All the patients had marked gamma-globulin changes (from 25·9 to 45·5 per cent.). Large numbers of L.E.-cells (up to 80 : 1,000) were found in four patients, and in two patients nuclei, corpuscles, and "rosettes" were found destroyed.

The average reaction with Antiserum No. 6006 in this group was +1·9 to 0·9 per cent. It was positive in five patients (range +3·1 to +5 per cent.), but negative in two (−0·6 and −6·3 per cent.), the second of whom was the only one in this group who had been treated with chloroquine for some years. One woman gave no reaction at all.

Discussion

Analysing the results of all three groups, we must note that lupus antigen, shown by reaction with Antiserum No. 6006, was found in the sera of 35 of the 44 patients.

Clinical correlation showed that the presence of lupus antigen in the serum is characteristic of the period of the active inflammatory process. Thus maximal reactions of +8 or +9 per cent. were obtained in the early stages of the disease or during exacerbations with pronounced synovitis, polyserositis, and rash. During "exacerbation" of the L.E. dermo-articular syndrome with pathological conditions of the internal organs, the antigen level decreased; this was manifested very clearly in acute cases with progressive and characteristic multiple symptoms (Fig. 2, overleaf).

During the progress of cases of diffuse renal disease, with the disappearance of the "peripheral" (skin and joint) signs, the reaction was negative with a significant decrease in gamma-globulin, as is clearly shown in Group 1, Sub-group iv. It may be supposed that the massive steroid hormone therapy and also probably treatment with chloroquine decreased the level of lupus antigen in Group 1, Sub-groups ii and iii. The influence of the character and degree of organ pathology on the level of lupus gamma-globulin was seen in Groups 2 and 3, i.e. in the patients with subacute and chronic disease. The Table shows that they were clinically more homogeneous than those in Group 1; the reaction results, especially in those with chronic disease, being more true to type.

Thus the differing content of lupus antigen in the acute cases was no doubt conditioned by the multiple symptoms of the clinical picture, with frequent involvement of the kidneys, and by the prolonged and massive steroid hormone therapy.

The diagnostic value of lupus antigen compared with the isolation of L.E.-cells is illustrated in Fig. 2, which shows that L.E. antigen is isolated comparatively frequently in patients with systemic lupus erythematosus, and can be of a definite diagnostic significance at any stage of the disease. During the subacute phase, the diagnostic signi-
ficance is particularly great; in six out of these eight patients this reaction was positive, although solitary L.E.-cells were found only in two of them.

However, the differential diagnosis of rheumatoid arthritis and subacute systemic lupus erythematosus is extremely difficult, and in this the study of the L.E.-cells gives no assistance, as a small number (1 to 2 per 1,000) can be found in cases of rheumatoid arthritis.

Studies of the lupus antigen during a period of exacerbation in sixteen patients with systemic lupus erythematosus of different types were also made. Fig. 3 shows that, in fourteen patients receiving active treatment, the lupus antigen level decreased despite the severity of the disease. Decreased or normal antigen levels were found chiefly in patients in a favourable phase of the disease; in those in whom the disease ran a continuous recurrent course in which the L.E. process could not be suppressed, the reaction remained unchanged.

Finally, a deepening of a negative reaction or a transition from a positive to a pronounced negative reaction was observed in patients with rapidly progressing renal disease. The study of the dynamics of the lupus antigen supports the idea that the antigen is characteristic of the acute phase of the lupus process. It is also probable that the level of lupus antigen corresponds to the general clinical tendency of the disease, and allows one to assess objectively both the prognosis and the efficiency of therapy.

**Summary**

(1) Antigen characteristic of systemic lupus erythematosus has been demonstrated in the sera of 35 out of 44 patients with active lupus disease by a method of paper electrophoresis.

(2) The level of the antigen reflects the acuteness of the pathological process, and a study of changes in the antigen level provides objective data on the progress of the disease, the efficiency of therapy, and the prognosis for individual patients.

(3) Steroid hormone therapy depresses the level of lupus antigen—probably by suppressing the activity of the disease process.

(4) The isolation of lupus antigen together with other laboratory indices assists the diagnosis of systemic lupus erythematosus, especially in subacute cases.

(5) In the course of severe L.E. nephritis, especially with renal failure, and in the presence of sclerotic and destructive changes in the internal organs, the lupus antigen could not be isolated.

**REFERENCES**


ELECTROPHORETIC DIAGNOSIS OF S.L.E.


**Diagnosty par l'électrophorèse du lupus érythémateux disseminé**

**Résumé**

1. Un antigène caractéristique de lupus érythémateux disséminé a été démontré dans les sérum de 35 sur 44 malades atteints de cette maladie en activité par la méthode d'électrophorèse sur papier.


3. La thérapie aux hormones stéroïdes déprime le niveau de l'antigène de lupus—probablement en supprimant l'activité du processus morbide.

4. L'isolement de l'antigène de lupus ainsi que d'autres indices de laboratoire aident le diagnostic du lupus érythémateux disséminé, particulièrement dans des cas subaigus.

5. Au cours d'une sévère nephrite due au lupus érythémateux, surtout en présence d'insuffisance rénale et d'altérations sclérotiques et destructives dans les organes internes, l'antigène de lupus ne pouvait pas être isolé.

**Diagnóstico por electroforesis del lupus eritematoso diseminado**

**SUMARIO**

1. Entre 44 enfermos padeciendo de lupus eritematoso activo, 35 mostraron poseer un antígeno característico de la enfermedad, evidenciado por el procedimiento de la electroforesis en papel.

2. El nivel del antígeno refleja la intensidad del proceso morbose y el estudio de las alteraciones de este nivel proporciona datos objetivos sobre la evolución de la enfermedad, la eficacia del tratamiento y la prognosis en enfermos individuales.

3. La terapéutica por las hormonas esteroides deprime el nivel del antígeno lúpico—probablemente porque suprime la actividad del proceso morbose.

4. El aislanieto del antígeno lúpico así como otros datos de laboratorio contribuyen al diagnóstico del lupus eritematoso diseminado, en particular en los casos subagudos.

5. En el curso de una nefritis grave debida al lupus eritematoso, en particular en presencia de insuficiencia renal y de alteraciones escleróticas y destructivas en los órganos internos, el antígeno lúpico no pudo ser aislado.
Diagnosis of Systemic Lupus Erythematosus by Electrophoresis

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