# Immunoelectrophoresis of Serum in "Lupoid" Hepatitis.*† (27325)

AVINOAM ZLOTNICK AND GERALD P. RODNAN‡ (Introduced by J. J. Vazquez)
*Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.*

"Lupoid" hepatitis, which represents a form of chronic hepatitis of young adults, is an increasingly well recognized entity, characterized by insidious onset of jaundice and hepatosplenomegaly preceded or accompanied by articular pain and other complaints common to systemic lupus erythematosus. The presence of extreme hypergammaglobulinemia is a prominent feature and in most cases there is a positive lupus erythematosus cell reaction. Several investigators have reported clinical and laboratory findings of this disease (1,2,3). The present study is concerned with the changes in serum proteins in this disease revealed by immunoelectrophoresis (IEP) and paper strip electrophoresis (PEP) and a comparison of these changes with those observed in Laennec's cirrhosis.

*Materials and methods.* Serum samples were obtained from 6 patients with chronic lupoid hepatitis and from 13 patients with Laennec's cirrhosis. The diagnosis of chronic lupoid hepatitis was established clinically and confirmed by liver biopsy in 4 instances; Laennec's cirrhosis was confirmed by biopsy in 6 cases. The sera of the patients with lupoid hepatitis were kept frozen at –20°C for several months prior to examination, while those of the patients with cirrhosis were kept frozen at –20°C for only 2 to 4 weeks.

Immunoelectrophoresis (IEP) was performed with the microapparatus of Scheidegger using 25 × 75 mm microscopic slides(4). A 2% agar solution prepared in sodium barbital buffer, pH 8.2, ionic strength 0.04 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 antiserum was introduced. Diffusion was allowed to take place for 24 hours and the slides were then washed and stained. Two slides were run for each sample; one was stained with Azocarmin G for protein and the other with Sudan IV for lipoprotein(5).

Five different antisera were used: 1) antiserum #511 from a horse immunized with pooled whole normal serum obtained from the Pasteur Institute in Paris; 2) Hyland IEP antiserum prepared by hyperimmunization of goats with human sera and selected fractions of human serum;§ 3) rabbit anti-alpha$_2$ macroglobulin serum obtained from Behringwerke in Marburg, Germany; 4) goat antihuman beta$_2$ macroglobulin serum obtained from Hyland;§ and 5) rabbit antihuman complement serum.§ All samples of chronic lupoid hepatitis were run against these 5 antisera and compared with normal control serum run parallel to the pathological serum. The sera from patients with Laennec's cirrhosis were run against Hyland IEP antiserum only.

Paper electrophoresis (PEP) was performed with a Spinco Model R apparatus, and the strips stained by alcoholic bromphenol blue were scanned in a Spinco Analytrol. Total serum protein was determined by a modified biuret method(6).

*Results.* Illustrations of the different precipitation lines of the normal and pathological sera obtained with antiserum #511 and with Hyland IEP antiserum are presented in Fig. 1 and 2. A detailed description of the morphology of these lines has been reported by Grabar(5) and others(7).

The results of the paper strip and immunoelectrophoretic examinations of the sera from patients with chronic "lupoid" hepatitis are indicated in Table I. *Albumin:* There was a

* Publication No. 6 of Division of Exp. Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

† Supported by grants from U.S.P.H.S. and Health Research Services Foundation of Allegheny Co., Pa.

‡ Dept. of Medicine, Univ. of Pittsburgh School of Med., Pittsburgh, Pa.

§ We wish to thank Dr. R. T. Fisk for kindly supplying us with samples of Hyland IEP antiserum and goat antihuman beta$_2$ macroglobulin serum, and Dr. Charles G. Cochrane for rabbit antihuman complement serum.

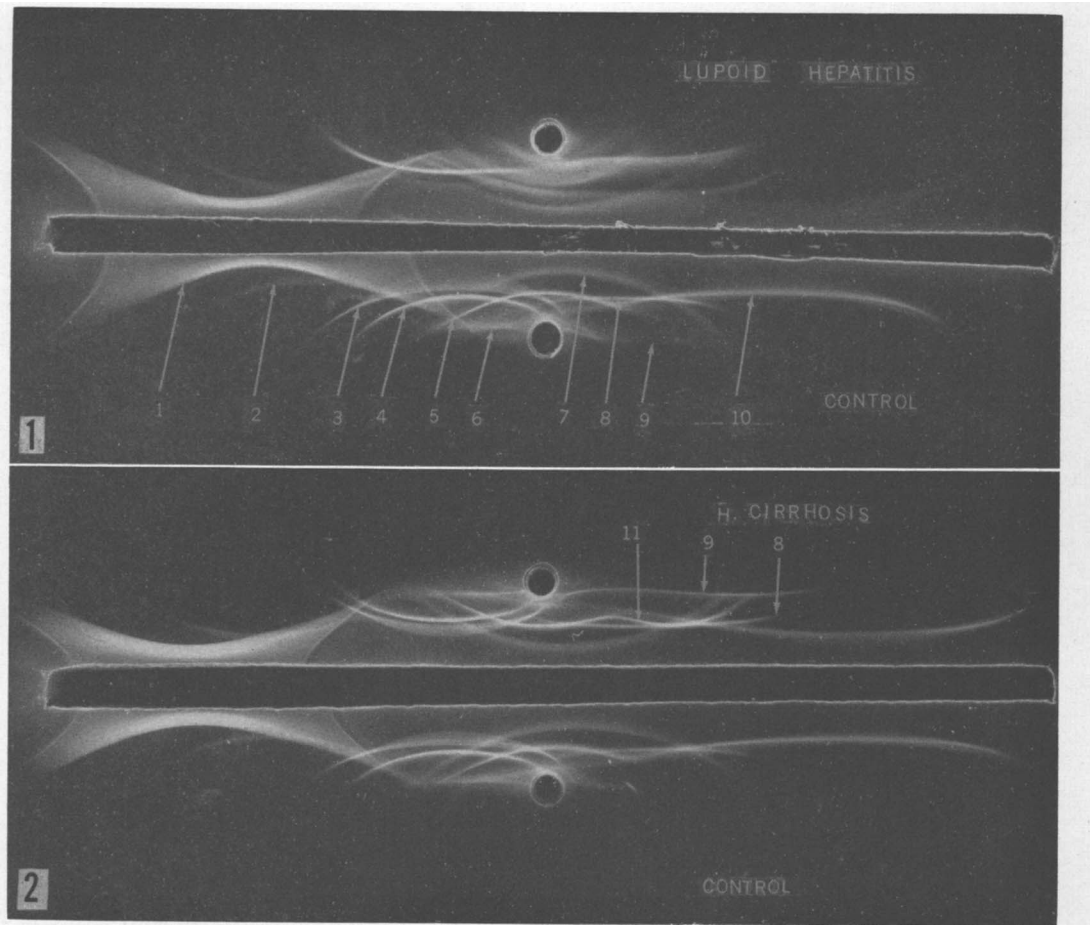


FIG. 1. Immunoelectrophoretic patterns of a patient with chronic "lupoid" hepatitis and a normal control. 1) Albumin, 2) $alpha_1$ globulin, 3) $alpha_2$ haptoglobin, 4) $alpha_2$ macroglobulin, 5) $beta_1$ A globulin, 6) $alpha_2$ and $beta_1$ lipoprotein, 7) siderophilin, 8) $beta_2$ A globulin, 9) $beta_2$ M globulin, 10) gamma globulin. Note in pathological serum the decrease in albumin $alpha_2$ haptoglobin, $alpha_2$-$beta_1$ lipoproteins, and $beta_1$ A globulin and the increase in $alpha_2$ M, $beta_2$ A and $beta_2$ M globulins. The gamma globulin is diffuse, indicating an antigen excess (IEP antiserum, Hyland).

FIG. 2. Immunoelectrophoretic patterns of a patient with hepatic cirrhosis and a normal control. Note presence of $beta_1$ C globulin (11) and increase of $beta_2$ A (8) and $beta_2$ M (9) globulins in the pathological serum (IEP antiserum, Hyland).

remarkable diminution of the albumin fraction in all cases, as seen in both paper strip and immunoelectrophoresis. *$Alpha_1$ globulin:* No significant changes were observed in the $alpha_1$ globulins. *$Alpha_2$ globulin:* The $alpha_2$ fraction appeared normal in amount on PEP, but on IEP there was a significant decrease in the $alpha_2$ haptoglobin in 4 patients and a very marked increase in the $alpha_2$ macroglobulin in all the patients. In one case, the $alpha_2$ macroglobulin had a double curvature, indicating a protein of 2 different electrophoretic mobilities but possessing one antigenic specificity. *Beta globulin:* The beta globulin fraction appeared normal in quantity and configuration on PEP. IEP revealed a number of changes, however. The $alpha_2$-$beta_1$ lipoprotein complex was diminished in all cases. This was particularly evident on slides stained with Sudan IV. The $beta_1$ A globulin was markedly diminished in 4 patients and the $beta_1$ C was absent in all. Dilution of the patient's serum did not alter these findings. The $beta_2$ M and the $beta_2$ A globulins were increased in all cases. *Gamma globulin:* This component showed a marked

TABLE I. Immuno- and Paper Strip Electrophoresis of Serum Proteins in 6 Patients with Chronic "Lupoid" Hepatitis.

| Patients' age, race and sex | Immunoelectrophoresis: Albumin | Globulins: $\alpha_1$ | $\alpha_2$ Hapt. | $\alpha_2$M Macr. | $\alpha_2$-$\beta_1$ Lipo. | $\beta_1$A | $\beta_1$C | $\beta_2$A | $\beta_2$M | $\gamma$ | Paper strip electrophoresis: Total prot. | Albumin | Globulins: $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ | L. E. reaction | Latex fixation | Liver biopsy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 W ♀ | ↓ | + | ↓ | ↑ | ↓ | + | 0 | ↑ | ↑ | A.E.* | 11.96 | 2.00 | .30 | .48 | .65 | 8.56 | Neg. | 1:640 | + |
| 15 W ♀ | ↓ | + | ↓ | ↑ | ↓ | ↓ | 0 | ↑ | ↑ | " | 8.59 | 1.99 | .10 | .26 | .41 | 5.83 | Neg. | Neg. | + |
| 12 W ♀ | ↓ | + | + | ↑ | ↓ | + | 0 | ↑ | ↑ | " | 7.03 | 1.20 | .27 | .34 | .74 | 4.49 | + | 1:40 | — |
| 17 W ♀ | ↓ | + | ↓ | ↑ | ↓ | ↓ | 0 | ↑ | ↑ | " | 7.97 | 1.75 | .27 | .24 | .55 | 5.16 | + | 1:2560 | + |
| 21 W ♀ | ↓ | + | + | ↑ | ↓ | ↓ | 0 | ↑ | ↑ | " | 9.26 | 2.94 | .30 | .77 | .77 | 4.48 | + | Neg. | + |
| 11 W ♀ | ↓ | + | + | ↑ | ↓ | ↓ | 0 | ↑ | ↑ | " | 7.63 | 2.47 | .21 | .34 | .62 | 3.99 | Neg. | Neg. | — |
| | | | | | | | | | | Normal range | 6.15 | 3.81 | .19 | .31 | .42 | .52 | | | |
| | | | | | | | | | | | 8.11 | 5.61 | .35 | .75 | .90 | 1.40 | | | |
| | | | | | | | | | | S.D. | .49 | .45 | .04 | .11 | .12 | .22 | | | |

\+ = Normal length, thickness and configuration of precipitin arc.
↑ = Increase in thickness and sometimes length of precipitin arc.
↓ = Decrease in thickness and length of precipitin arc.
0 = Absent.

* A.E. = Antigen excess.

TABLE II. Immuno- and Paper Strip Electrophoresis of Serum Proteins in 13 Patients with Hepatic Cirrhosis.

| Patients' age, race and sex | Immunoelectrophoresis: Albumin | Globulins: $\alpha_1$ | $\alpha_2$ Hapt. | $\alpha_2$M Macr. | $\alpha_2$-$\beta_1$ Lipo. | $\beta_1$A | $\beta_1$C | $\beta_2$A | $\beta_2$M | $\gamma$ | Paper strip electrophoresis: Total prot. | Albumin | Globulins: $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ | L. E. reaction | Latex fixation | Liver biopsy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 43 W ♂ | + | + | + | ↑ | + | + | + | ↑ | ↑ | A.E.* | 9.75 | 3.83 | .28 | .34 | 1.01 | 4.28 | Neg. | Neg. | + |
| 36 W ♂ | + | + | + | + | + | + | + | ↑ | ↑ | " | 8.7 | 4.48 | .35 | .57 | .79 | 2.51 | Neg. | Neg. | — |
| 47 W ♂ | ↓ | + | + | + | + | + | + | ↑ | ↑ | " | 7.42 | 1.39 | .38 | .57 | 1.14 | 3.93 | Neg. | Neg. | + |
| 48 W ♂ | + | + | + | + | + | + | + | ↑ | ↑ | + | 7.56 | 3.75 | .36 | .66 | .77 | 2.03 | Neg. | Neg. | + |
| 34 W ♂ | + | + | + | + | + | + | + | ↑ | 0 | A.E. | 7.37 | 3.69 | .31 | .55 | 1.16 | 1.66 | Neg. | Neg. | + |
| 42 W ♂ | + | + | + | + | + | + | + | ↑ | ↑ | + | 8.06 | 4.14 | .28 | .73 | 1.10 | 1.90 | Neg. | Neg. | + |
| 43 W ♂ | ↓ | + | + | + | + | + | + | ↑ | ↑ | + | 7.22 | 2.17 | .25 | .51 | .58 | 3.70 | Neg. | Neg. | — |
| 62 W ♂ | + | + | + | + | + | + | + | ↑ | ↑ | + | 6.1 | 2.03 | .25 | .57 | .77 | 2.38 | Neg. | Neg. | — |
| 48 W ♂ | ↓ | + | ↓ | + | + | + | + | ↑ | ↑ | + | 6.4 | 2.07 | .32 | .41 | —3.60— | | Neg. | Neg. | — |
| 57 W ♂ | + | + | + | + | + | + | + | ↑ | ↑ | + | 7.2 | 3.5 | .30 | .7 | .5 | 2.2 | Neg. | Neg. | — |
| 55 W ♂ | + | + | ↓ | + | + | + | + | ↑ | ↑ | + | 5.5 | 2.96 | .18 | .32 | .42 | 1.62 | Neg. | Neg. | + |
| 54 W ♂ | + | + | ↓ | + | + | + | + | ↑ | ↑ | + | 6.7 | 3.3 | .34 | .51 | .89 | 1.66 | Neg. | Neg. | — |
| 39 W ♂ | + | + | + | + | + | + | + | ↑ | ↑ | + | 6.7 | 3.51 | .24 | .52 | .81 | 1.62 | Neg. | Neg. | — |

See footnotes, Table I.

TABLE III. Comparison of Alteration in Serum Proteins in Chronic "Lupoid" Hepatitis, Laennec's Cirrhosis and SLE Revealed by Paper Strip Electrophoresis and Immunoelectrophoresis.

| Serum protein component | Chronic "lupoid" hepatitis | Laennec's cirrhosis | Systemic lupus erythematosus* |
|---|---|---|---|
| Albumin | ↓↓ | ↓ | ↓ |
| *Alpha globulins* | | | |
| $\alpha_1$ | + | + | + |
| $\alpha_2$ Haptoglobin | ↓ 4 out of 6 | ↓ 3 out of 13 | ↑ |
| $\alpha_2$ Macroglobulin | ↑ | + | |
| $\alpha_2$ Lipoproteins | ↓↓ | + | |
| *Beta globulins* | | | |
| $\beta_1$ Siderophilin | + | + | + |
| $\beta_1$ Lipoprotein | ↓ | + | |
| $\beta_1$ A Globulin | ↓ | + | ↓ |
| $\beta_2$ A Globulin | ↑ | ↑ | ↑ |
| $\beta_2$ M Globulin | ↑ | ↑ | ↑ 50% of cases |
| *Gamma Globulin* | ↑↑↑ | ↑↑ | ↑–↑↑ |

+ = Present in apparently normal amounts.
↓ = Decreased.
↑ = Increased.
* Data obtained from Seligman(11).

increase on PEP and appeared as a broad-based rounded peak which would appear to rule out the presence of a single paraprotein. On IEP, the gamma globulin was revealed as a broad hazy band, which sometimes diffused into the trough, indicating an antigen excess. A sharp line could be obtained when the serum was diluted 1:4.

The results of the paper strip and the immunoelectrophoresis examinations of the sera from patients with Laennec's cirrhosis are given in Table II. There were no significant changes in the albumin, alpha$_1$-beta$_1$ lipoproteins and the beta$_1$ A and beta$_1$ C globulins. In 4 patients, the alpha$_2$ haptoglobin was diminished. The amount of beta$_2$ A and beta$_2$ M globulin fractions was increased in all patients. The gamma globulin appeared as a hazy broad band in 5 cases, indicating antigen excess, while in other cases did not differ from the controls. The pertinent changes observed in PEP were a diminution of the albumin fraction and increase in the gamma globulin fraction. Separation of the gamma globulin peak from the beta globulin peak was sometimes difficult if not impossible.

Comparison of the changes in composition of the serum proteins in chronic "lupoid" hepatitis and Laennec's cirrhosis is given in Table III.

*Discussion.* The alterations in composition of the serum proteins in chronic "lupoid" hepatitis determined by paper strip electrophoresis consisted of a diminution in the albumin fraction and a marked increase in the gamma globulin. Immunoelectrophoresis has revealed a number of more specific abnormalities in alpha and beta globulins. The alpha$_2$ fraction, which did not appear unusual on paper electrophoresis, showed remarkable changes on immunoelectrophoresis. There was a decrease in alpha$_2$ haptoglobin and an increase in alpha$_2$ macroglobulin fractions. The decrease in haptoglobin fraction is in accord with Nyman(8) who reported a decrease in the chemically determined haptoglobin in postnecrotic and Laennec's cirrhosis. An increase in the alpha$_2$ macroglobulin fraction has been observed in scleroderma and rheumatoid arthritis(7), but has not been reported previously in liver disease. In one of the cases of chronic "lupoid" hepatitis, the precipitin line of the alpha$_2$ macroglobulin had a double curvature, a configuration resembling that of the beta$_2$ M globulin in some cases of macroglobulinemia of Waldenstrom (9). The significance of the double curvature and of the overall increase of this protein in these patients is not known. Whether it be the result of altered liver cell function or of a hyperactive response of the reticuloendothelial system remains to be determined.

The decrease in number and intensity of the lines of the beta$_1$ complex, which also appears to be a characteristic feature of the sera from patients with "lupoid" hepatitis, has also been described in some cases of fatal hepatitis of viral origin(5). Of particular interest, however, is the decrease of beta$_1$ A globulin and the absence of the beta$_1$ C fraction in most of our patients with "lupoid" hepatitis. The absence of the beta$_1$ C globulin could be attributed to the aging of the serum and transformation of this globulin into the beta$_1$ A globulin(10).

The beta$_1$ A globulin has been identified with one of the components of complement (10) and its decrease has been described as a

characteristic feature of systemic lupus erythematosus(11). The decrease of this protein in both "lupoid" hepatitis and SLE suggests that a common immunologic process might be operating in these pathological entities. It is thus tempting to postulate that the decrease of $beta_1$ A globulin in "lupoid" hepatitis is due to its utilization, perhaps by antigen-antibody complexes in a similar way as in SLE. Although not established in these studies, the above results could suggest that "lupoid" hepatitis is a variant of systemic lupus erythematosus.

The increase in $beta_2$ A and $beta_2$ M proteins has been reported in a variety of diseases (5) and is not specific for liver disease alone.

From this study it is evident that immunoelectrophoresis may add interesting information to the variations in composition of the serum proteins in "lupoid" hepatitis. The decrease in the albumin and the increase in gamma globulin fraction are identical on both PEP and IEP. However, the $alpha_2$ and the beta fractions, which do not show significant changes in PEP, reveal alterations when separated by IEP. The decrease in $alpha_2$ haptoglobin and the increase in the $alpha_2$ macroglobulin may compensate each other and appear as a normal total $alpha_2$ globulin on PEP. The same might be true for the $beta_1$ and the $beta_2$ fractions.

Comparison of the changes in composition of the serum proteins in chronic "lupoid" hepatitis and Laennec's cirrhosis observed in the present study and those reported in systemic lupus erythematosus are summarized in Table III. Some of these variations are probably matters of degree rather than kind and may reflect a state of, at least temporarily, more severe liver damage in the patients with chronic hepatitis compared to those with cirrhosis. However, the decrease of $beta_1$ A globulin seems to be more specific and is suggestive of a close relationship between "lupoid" hepatitis and systemic lupus erythematosus.

*Summary.* Immuno- and paper strip electrophoresis of serum proteins were performed on 6 patients with "lupoid" hepatitis and compared with the findings in 13 patients with Laennec's cirrhosis. The following changes were observed in "lupoid" hepatitis: There was a decrease in albumin, $alpha_2$ haptoglobin, $alpha_2$-$beta_1$ lipoproteins and $beta_1$ A globulin and an elevation in $alpha_2$ M, $beta_2$ A, $beta_2$ M and gamma globulins. Immunoelectrophoresis in Laennec's cirrhosis revealed only an increase in $beta_2$ A, $beta_2$ M and gamma globulins. Paper strip electrophoresis revealed in both diseases a decrease in the albumin fraction and an increase in gamma globulins. The significance of these alterations in the serum proteins is discussed. The $beta_1$ A globulin has been identified with one of the components of the complement and is diminished both in SLE and in "lupoid" hepatitis. This suggests that a similar immunologic process is operating in both diseases.

The authors wish to thank Mrs. M. Courville and Miss Gail H. Jones for their technical assistance.

1. Bearn, A. G., Kunkel, H. G., Slater, R. J., *Am. J. Med.*, 1956, v21, 3.

2. Cattan, R., Carasso, R., Vesin, P., Burtin, P., *Revue Intern. Hepatol.*, 1957, v7, 33.

3. Willcox, R. G., Isselbacher, K. J., *Am. J. Med.*, 1961, v30, 185.

4. Scheidegger, J. J., *Intl. Arch. Allergy*, 1955, v7, 103.

5. Grabar, P., Burtin, P., *Analyse Immunoelectrophoretique*, Paris: Masson et Cie, 1960.

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

7. Zlotnick, A., Rodnan, G. P., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 112.

8. Nyman, M., *Scand. J. Clin. Lab. Invest.* 1959, Suppl. 39, 115.

9. Roulet, D. L. A., Spengler, G. A., Gugler, E., Bütler, R., Ricci, C., Riva, G., Hassig, A., *Helvetica Med. Acta*, 1961, v28, 136.

10. Muller-Eberhard, H. J., Nilsson, U., *J. Exp. Med.*, 1960, v111, 217.

11. Seligmann, M. in Grabar and Burtin, *Analyse Immunoelectrophoretique*. Paris: Masson et Cie., p207, 1960.

Received December 27, 1961. P.S.E.B.M., 1962, v109.