BIKEN JOURNAL Vol. 10, 175-188, 1967

IMMUNOELECTROPHORETIC STUDIES ON β_{1E}-GLOBULIN IN HUMAN SERUM[1]

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(Received June 8, 1967)

SUMMARY Highly purified human fourth component of complement (C'4) which showed a single precipitin line against horse antihuman serum in the β_1-globulin region was used for identification of the precipitin line of β_{1E}-globulin (β_{1E}-line) in the immunoelectrophoretic pattern of fresh human serum. Identification was achieved by a combination of immunoelectrophoresis and Ouchterlony's double diffusion technique. Differentiation of the β_{1E}-line from other lines in the β_1-globulin region has been facilitated by the use of anti $\beta_{1C/1A}$ serum and specific absorbed horse antihuman serum.

In the immunoelectrophoretic pattern of normal human serum, the β_{1E}-line ran parallel with the $\beta_{1C/1A}$-globulin line ($\beta_{1C/1A}$-line) from the antigen well to the β_1-globulin region and crossing of the two lines was never seen.

It was also shown that the β_{1E}-line of normal human serum changed in appearance, and crossing of the β_{1E}-line and $\beta_{1C/1A}$-line was observed after hydrazine treatment or addition of immune precipitate.

A close relationship between the serum C'4 level and the appearance of the β_{1E}-line in the serum was demonstrated. It is interesting to note that the β_{1E}-line was indistinct or absent when the C'4 level of the serum was difinitely low.

INTRODUCTION

In the previous paper (INAI *et al.*, 1967a), it was reported that the serum complement level was decreased in some cases in various diseases, and that a markedly decreased or zero C'4 level was also found in these conditions.

In 1963, Müller-Eberhard and Biro reported the isolation of a new protein fraction as the result of purification of C'4 from human serum. This was identified as β_{1E}-globulin by immunoelectrophoretic analysis. However, in their report, the differentiation of the β_{1E}-line

1 Parts of this work were reported at the Third Symposium on Complement in August 1966 at Hakone, Japan, and at the 16th Annual Meeting of the Japanese Society of Allergy in October 1966 at Tokyo, Japan.

from other precipitin lines in the β_1-globulin region was not clear from the photograph of the immunoelectrophoretic pattern of normal human serum. Moreover, it is still uncertain whether C'4 actually corresponds with β_{1E}-globulin. There have been numerous reports of immunoelectrophoretic analyses of sera of patients with various diseases, but no reports on the relationship between the serum C'4 level and the β_{1E}-line as far as we know. FOSTIROPORLOS *et al.* (1965) investigated the synovial fluid in patients with various joint diseases and reported on the alteration of β_{1E}-globulin, but they did not mention the relation between the C'4 level and β_{1E}-globulin.

In the previous paper (INAI *et al.*, 1967b), we reported a new method for purifying C'4 which combined several methods that were effective in eliminating the contaminating C'1 inactivator. Immunoelectrophoretic analysis of purified C'4 gave a single precipitin line and this line corresponded to the line of β_{1E}-globulin which was obtained with horse antihuman serum.

The present study was to determine the situation of the precipitin line of purified C'4 and to distinguish it from other lines of normal human serum by immunoelectrophoresis. The relation between the decrease of C'4 activity in patients' sera and the alteration of the precipitin line of β_{1E}-globulin was also investigated.

MATERIALS AND METHODS

1. *Purification of C'4*

The procedure for purification of C'4 from fresh human serum was described in the previous paper (INAI *et al.*, 1967 a).

2. *Sera*

As a control in immunoelectrophoresis, sera were obtained from many healthy people, they were pooled and stored at $-20°C$ until use. In the clinical investigation, sera of randomly selected persons with rheumatoid arthritis, cirrhosis of liver and multiple myeloma were obtained and stored at $-20°C$ until use. These serum specimens were not stored for more than 3 weeks.

3. *Antisera*

1) Horse antihuman serum

This was prepared at the Osaka Microbial Diseases Research Foundation (Lot No. 2), and was used for immunoelectrophoresis and Ouchterlony's double diffusion technique in this study.

2) Partially purified horse antihuman serum

As horse antihuman serum has remarkable anticomplementary activity, elimination of this activity was attempted.

To 4.7 ml of this antiserum was added an equal volume of saturated ammonium sulphate. After four hours at room temperature, the precipitate was separated by centrifugation at 8,000 rpm at 0°C for 15 minutes and dissolved in deionized water. This solution was dialysed overnight against tapping water and for 24 hours against deionized water. Then, this material was dialysed overnight against Tris-phosphoric acid buffer of pH 8.4, conductance 1.2×10^3 micromhos. The dialysate was centrifuged at 4,000 rpm at 0°C. The supernatant was applied to a 1.5×18 cm column of DEAE cellulose previously equilibrated with Tris-phosphoric acid buffer of pH 8.4, conductance 1.2×10^3 micromhos. The column was thoroguhly washed with this buffer of 1.2×10^3 micromhos conductance, then with buffer of 1.95×10^3 micromhos, and then with buffer of 3.3×10^3 micromhos conductance. The fraction which was eluted with Tris-phosphoric acid buffer of 3.3×10^3 micromhos conductance was collected and concentrated to its original volume by ultrafiltration. This fraction corresponded to T-globulin and exhibited the same precipitin lines with normal human serum as the original antiserum on immunoelectrophoresis, and its anticomplementary activity had been almost completely eliminated.

3) Absorbed horse antihuman serum

As described in the previous paper (INAI *et al.*, 1967 b), horse antihuman serum was absorbed with the fractions of human serum which did not include C'4 and β_{1C}-globulin.

4) Rabbit antihuman C'4 serum

Five ml of purified C'4 (total protein content, 3.26 mg) in complete Freund's adjuvant (containing 1 mg dried tubercle bacilli, 4.25 ml of Drackeol No. 6 and 0.75 ml of Aracel A) was divided into three equal portions and each was injected into the foot pad and intracutan of the back of 3 rabbits. Animals were bled one month after the injection.

The sera of these animals were frozen at −20°C without inactivation. These sera gave a single precipitin line not only with isolated C'4 but also with normal human serum upon immunoelectrophoresis and by Ouchterlony's double diffusion technique.

5) Goat antihuman $\beta_{1C/1A}$-globulin serum

This antiserum produced by Hyland Laboratory was used for differentiation of the β_{1E}-line from the $\beta_{1C/1A}$-line.

4. *Materials used for immune hemolysis in gels*

All diluents and preparation of sheep erythrocytes (E), amboceptor (A), sensitized sheep erythrocytes (EA), guinea pig C'1 (C'1 gp), guinea pig C'2 (C'2 gp), EDTA treated guinea pig complement (C'-EDTA) and EA with C'1 gp activity (EAC'1 gp) were as reported previously (NAGAKI *et al.*, 1967 a). Titration of C'H50, C'1 and C'4 was done as described in the previous report (INAI *et al.*, 1967 a).

5. *Immunoelectrophoresis*

Ten ml of 1 per cent Oxoid Ionagar No. 2 in veronal-HCl buffer (μ=0.05, pH 8.6) was layered over a photographic glass slide (7.5×12 cm) and then allowed to gelatinize. An antigen well (diameter 2 mm) was punched out at a point 3 cm from the cathodal end. This well was filled with pooled human serum or purified human C'4. The electrode reservoirs contained veronal-HCl buffer (μ=0.05, pH 8.6). Electrophoresis was carried out at 4°C for 1.5 hours at 6 v/cm. Under these conditions, the spot indicating the albumin fraction moved to the anodal side about 2.5 cm from the antigen well, while the β_1-globulin fraction did not move.

After electrophoresis, an antibody trough (2 mm width) was made with a razor blade. The distance between the edge of the trough and the well was usually 4 mm. Horse antihuman serum was put into the trough, and the slide was kept in a moist chamber at room temperature overnight.

After this procedure, precipitin lines were visualized, and the plate was washed for one day in a large volume of physiological saline solution, and then dried at 37°C, and stained with 0.1 per cent Amidoblack 10 B solution.

RESULTS

1. *Hemolytic activity of C'4 after electrophoresis on agar plate*

The situation of purified C'4 on the agar plate after electrophoresis was studied.

In this experiment, C'4 with an activity of about 20×10^{12} effective molecules (eff. mol.) per ml was applied in a volume of 0.03 ml. The electrophoresis of C'4 on agar gel was performed as follows; a glass slide (7.5×2.5 cm) was coated with a layer of 1 per cent Difco Special Agar Noble in veronal HCl buffer (μ=0.05, pH 8.6) which was allowed to gelatinize. Then, a well (diameter 2 mm) was punched out in the center of this slide, and the C'4 was put into this well. Electrophoresis was carried out at 4°C for 1.5 hours, at 6 v/cm.

To detect the hemolytic activity of C'4 to EAC'1 on the agar gel, 4 ml of 1 per cent Difco Special Agar Noble, 4 ml of EAC'1 cells (7.5×10^8 cells per ml), 4 ml of C'2 (600 eff. mol. per cell) and 0.2 ml of 1 per cent DEAE-Dextran were mixed at 50°C. The C'4 on the agar was subjected to electrophoresis and then the above mixture was spread thinly on this agar plate. The plate was incubated at 37°C for 20 minutes and then it was treated with 12.5 fold of C'EDTA for 2 hours at 37°C.

As shown in Fig. 1, the hemolytic zone was visualized as an oval shaped spot on the anodal side from the original point. Namely, the position of the hemolytic zone which was produced by electrophoresis of hemolytically active C'4 was in the β_1-globulin region.

2. *Immunoelectrophoretic pattern of purified C'4*

To investigate the purity of the purified C'4, immunoelectrophoretic analysis was performed with horse antihuman serum.

Volumes of 0.03 ml of whole human serum and purified C'4 (48.5×10^{12} eff. mol. per ml, protein content 2 mg per ml) were filled in the wells. After electrophoresis, horse antihuman serum was put into the troughs. As shown

in Fig. 2, a single precipitin line between C'4 and antiserum was seen in the β_1-globulin region. This single line of C'4 might correspond to β_{1E}-globulin, as reported by MÜLLER-EBERHARD and BIRO (1963).

On the other hand, as shown in Fig. 3, when rabbit antihuman C'4 serum was used on immunoelectrophoresis of C'4 and normal human serum, this antiserum reacted and a single precipitin line developed with both antigens. This antiserum against human C'4 had high neutralizing activity for the hemolytic activity of C'4 and high agglutinating activity for the EAC'1, 4 cells (NAGAKI *et al.*, 1967b). These results showed that the C'4 used in the following experiments was pure.

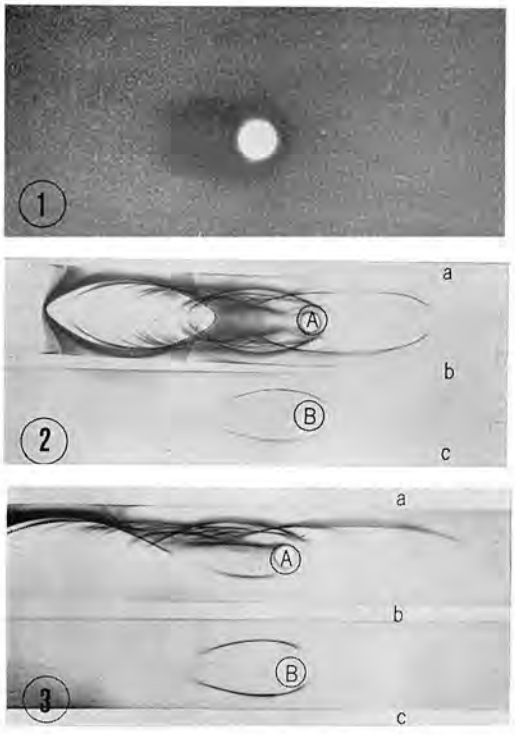


FIGURE 1 Hemolytic activity of C'4 after electrophoresis on agar plate. C'4 was subjected to electrophoresis on an agar plate, and then the hemolytic reaction was carried out on this plate.

FIGURE 2 Immunoelectrophoretic pattern of purified C'4.
A: normal human serum B: purified C'4
a, b and c: horse antihuman serum

FIGURE 3 Immunoelectrophoretic pattern of normal human serum and purified C'4 against rabbit anti C'4 serum.
A: normal human serum B: purified C'4
a and c: horse antihuman serum
b: rabbit antihuman C'4 serum

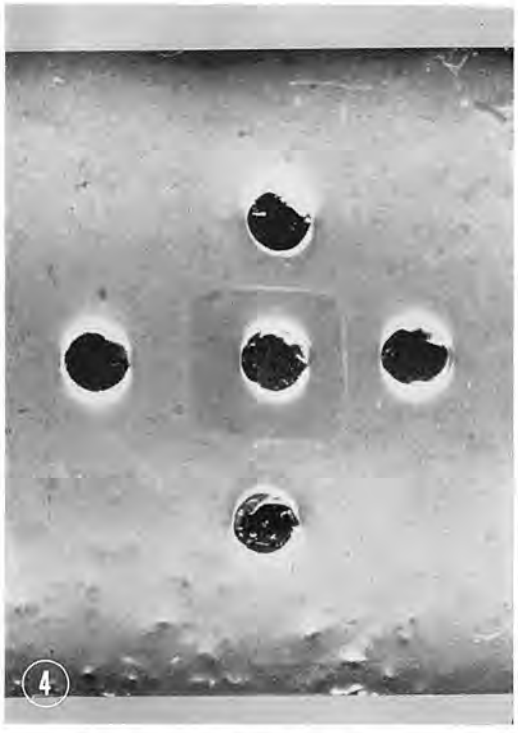


FIGURE 4 Lysis of EAC'1 cells by purified C'4 in diffusion plate.
The central well was filled with purified C'4 and the four peripheral wells were filled with horse antihuman serum. The distances from the central well to the peripheral wells were 2 mm, 3 mm, 4 mm and 5 mm respectively. The hemolytic zone is limited by a fused precipitin line.

3. *Lysis of EAC'1 cells by purified C'4 in the diffusion plate*

The following experiments were done to test whether so-called β_{1E}-globulin actually corresponds to C'4 itself.

Four ml of 2.5 per cent Oxoid Ionagar No. 2, 4 ml of 1.5×10^8 EAC'1 cells and 4 ml of C'2 (to supply 600 eff. mol. per cell) were mixed at 50°C, and 0.2 ml of a 1 per cent solution of DEAE-Dextran was added to this mixture. This was poured on the glass slide (7.5×2.5 cm) and gelatinized. The distances between the central well and peripheral wells were 2 mm, 3 mm, 4 mm and 5 mm respectively. The central well was filled with 5.5×10^{12} eff. mol. per ml of C'4 (total protein content, 0.22 mg per ml) and the peripheral wells were filled with partially purified horse antihuman serum. As described in MATERIALS AND METHODS, the anticomplementary activity of horse serum had been almost completely eliminated from this purified horse antihuman serum.

After a faint precipitin line had appeared on incubation for 8 hours at room temperature, a 12.5 fold of C'EDTA was poured onto the plate. Hemolysis of EAC'1 cells and precipitin lines were observed after incubation at 37°C for 2 hours and then at 30°C overnight.

As shown in Fig. 4, the precipitin lines formed between C'4 and horse antihuman serum in the peripheral wells fused with each other. A hemolytic zone was formed from the edge of the central well to the precipitin lines. The spread of this hemolytic zone was completely stopped by the precipitin line. It is conceivable that anti-β_{1E} antibody in antiserum reacted with C'4 (β_{1E}-globulin), and

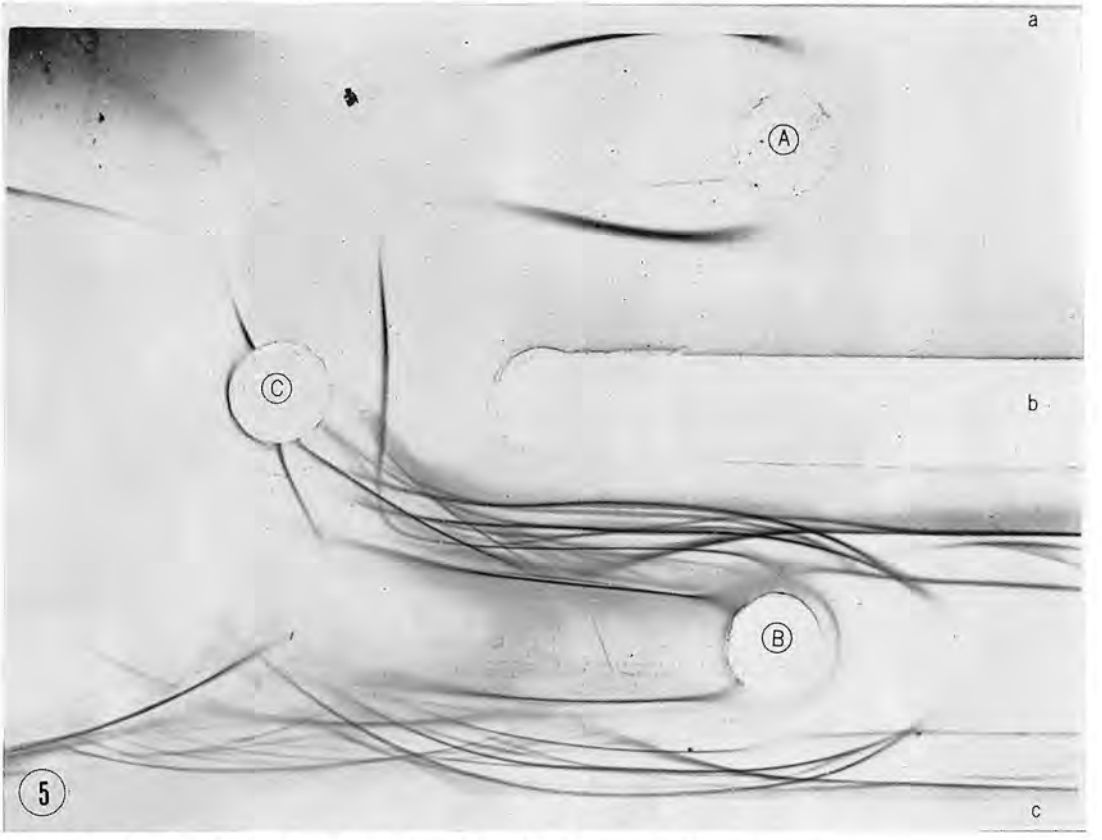


FIGURE 5 Immunoelectrophoretic situation of β_{1E}-globulin in normal human serum.
C'4 and normal human serum were put into wells A and B. After electrophoresis, C'4 was put into well C and horse antihuman serum into troughs a, b and c.

the hemolytic activity of C'4 was neutralized.

4. *Immunoelectrophoretic situation of the precipitin line of β_{1E}-globulin in immunoelectrophoresis of normal human serum*

To examine the precipitin line of C'4 in normal human serum, Ouchterlony's double diffusion technique was combined with immunoelectrophoresis.

Normal human serum and 7.5×10^{12} eff. mol. per ml of C'4 (total protein content, 0.35 mg per ml) were subjected to electrophoresis as described above, and allowed to reacted with horse antihuman serum. At the same time C'4 was allowed to diffuse in the plate. As shown in Fig. 5, the precipitin line formed between C'4 and antiserum fused with the precipitin line in the β_1-globulin region. This precipitin line seems to correspond to the line of so-called β_{1E}-globulin.

5. *Immunoelectrophoresis of normal human serum by use of absorbed horse antihuman serum*

It is difficult to distinguish the β_{1E}-line from other lines in the β_1-globulin region, and it is particularly difficult to distinguish the β_{1E}-line from the $\beta_{1C/1A}$-line. Therefore, absorbed horse antihuman serum and goat antihuman $\beta_{1C/1A}$-globulin serum were applied in the same experiments. C'4 and normal human serum were subjected to electrophoresis and allowed to react with these antisera.

As shown in Fig. 6, when this absorbed antiserum was used on immunoelectrophoresis of normal human serum, the lines of transferrin, β_{2A}-globulin and a part of the slowly migrating α_2-globulin disappeared. Then the lines in the β_1-globulin region became clear.

As shown in Fig. 7, the precipitin lines of β_{1E}-globulin and $\beta_{1C/1A}$-globulin were more easily identified. Crossing of the β_{1E}-line and $\beta_{1C/1A}$-line was never seen in normal human serum.

6. *Effect of Hydrazine on the β_{1E}-line*

Using immunoelectrophoresis, MÜLLER-EBERHARD and BIRO (1963) showed that

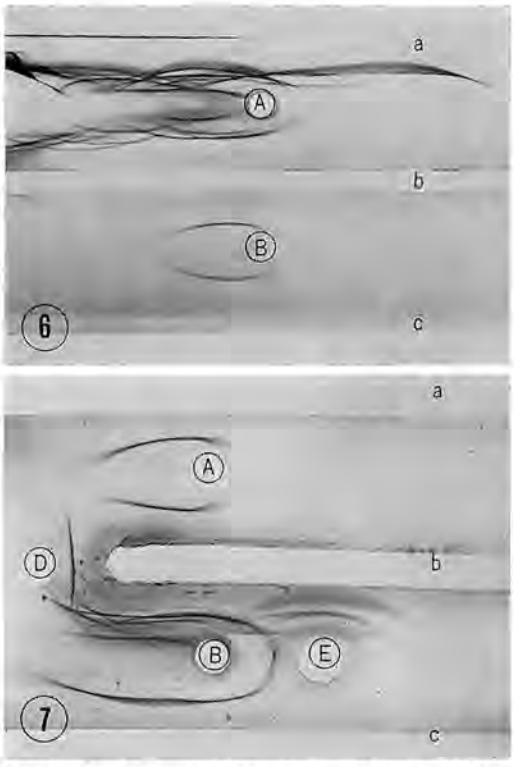


FIGURE 6 Immunoelectrophoresis of normal human serum using absorbed horse antihuman serum.
A: normal human serum B: purified C'4
a and c: unabsorbed horse antihuman serum
b: absorbed horse antihuman serum

FIGURE 7 Differentiation of the β_{1E}-line from the $\beta_{1C/1A}$-line using absorbed horse antihuman serum.
Purified C'4 in well A and normal human serum in well B were subjected to electrophoresis. After electrophoresis, horse antihuman serum was put into troughs a and b, goat antihuman $\beta_{1C/1A}$-globulin serum was put into trough c and well E, and purified C'4 was put into well D.

hydrazine treatment of C'4 first resulted in an increased electrophoretic mobility of the β_{1E}-line and then after a few minutes caused a marked decrease in its mobility.

In this experiment, alteration of the β_{1E}-globulin was observed using normal human serum instead of purified C'4.

Five ml of normal human serum was mixed with 1.25 ml of 0.075 M hydrazine, and this mixture was incubated at 37°C. At suitable

intervals (10, 20, 30 and 60 minutes), 1.25 ml of this mixture was withdrawn and neutralized with 0.25 ml of 0.075 M HCl. In these experiments, scarcely any C'4 activity was detected even after 10 minutes. These samples and nontreated serum were subjected to electrophoresis. After electrophoresis antiserum was put into the troughs.

As shown in Fig. 8, on treatment with hydrazine, the β_{1E}-line gradually extended to the cathodal side with time and crossed the $B_{1C/1A}$-line. No change was found on the anodal side, as C'4 activity had been lost after 30 minutes treatment.

7. *Effect of immune precipitate on the β_{1E}-line*

Peetoom and Pondman (1963) reported that the immunoelectrophoretic pattern of β_{1E}-globulin separated into β_{1E1} and β_{1E2} when normal human serum was treated with immune precipitate (Ea/anti-Ea). An experiment was performed to confirm this.

As the immune precipitate, egg albumin and anti-egg albumin specific precipitate were used. Volumes of 7.5 ml, 6.0 ml, 3.0 ml and 1.5 ml of suspension of immune precipitate were introduced into 4 tubes and centrifuged and the supernatants were discarded. To these tubes, 1.5 ml of pooled human sera were added and tubes were incubated for 30 minutes at 30°C and centrifuged. Then the C', C'1 and C'4 in each supernatant were measured. As a control, a tube was set up containing 1.5 ml of pooled human serum without added immune precipitate.

The C'H50, C'1H63 and C'4H63 in these treated sera and in control serum are shown in Table 1. Sample No. 3 contained the least C'1H63 and C'4H63. The causes of the discrepancy between the total N in the immune precipitate used for treatment of serum and the decreases in C'1H63 and C'4H63 is uncertain.

These samples and control sera were used for immunoelectrophoretic analysis. As shown in Fig. 9, with treated serum the β_{1E}-line crosses the $\beta_{1C/1A}$-line, and the β_{1C}-line is converted to the β_{1E}-line. However, transfer of the β_{1E}-line to the cathodal or anodal side and formation of lines β_{1E1} and β_{1E2} were not observed.

8. *Immunoelectrophoretic pattern of the β_{1E}-line in the sera of various patients*

The relationship between the activity of C'4 and the β_{1E}-line of the serum in various diseases was investigated.

Sera were subjected to electrophoresis on an agar plate, and allowed to reacted with horse antihuman serum, absorbed horse antihuman serum or rabbit antihuman C'4 serum.

Results of these experiments were summarized in Table 2.

These results show a close relationship between the C'4 levels and the appearance of the β_{1E}-line in the serum in many cases. Thus, the β_{1E}-line appeared normal when the C'4 level was normal, and was not present when the C'4 level was low or apparently zero. In a few cases, as in case 3 on Aug. 10, 1966 (Fig. 13) and case 4 (Fig. 15), the β_{1E}-line was barely formed, and as in case 3 on Dec. 29, 1966 (Fig. 14), the C'4 level was apparently zero but the β_{1E}-line was only slightly decreased.

TABLE 1 *C' and its components in serum specimens treated with immune precipitate*

Sample	Total N in immune precipitate used (γ)	C'H50	C'1H63	C'4H63
1	1,500	3.4	35,500	21,500
2	1,200	4.2	10,000	14,000
3	600	6.3	7,800	7,600
4	300	16.4	88,000	63,000
Control	0	66.3	165,000	155,000

FIGURE 8 Effect of Hydrazine on the β_{1E}-line.

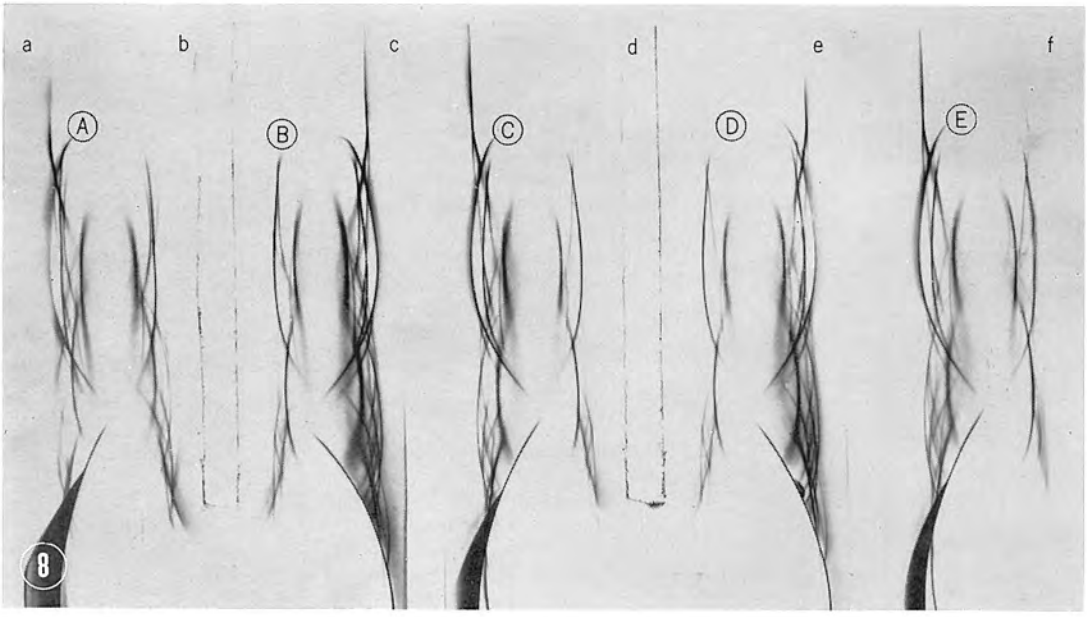
The immunoelectrophoretic patterns of normal human serum treated with hydrazine are shown. Control serum (untreated serum in well A) and treated sera (treated for 10, 20, 30 and 60 minutes in wells B, C, D and E, respectively) were subjected to electrophoresis. After electrophoresis, absorbed horse antiserum and unabsorbed horse antiserum were put into the troughs from a to f alternatively. The β_{1E}-line extended to the cathodal side with time and crossed the $\beta_{1C/1A}$-line.

FIGURE 9 Effect of immune precipitate on the β_{1E}-line.

The immunoelectrophoretic patterns of normal human serum treated with immune precitpitate are shown.

The β_{1E}-line crossed the $\beta_{1C/1A}$-line, and the conversion of β_{1C}-line to β_{1A}-line was observed.

C : control serum
1 : sample No. 1
2 : sample No. 2
3 : sample No. 3
4 : sample No. 4
troughs : absorbed horse antihuman serum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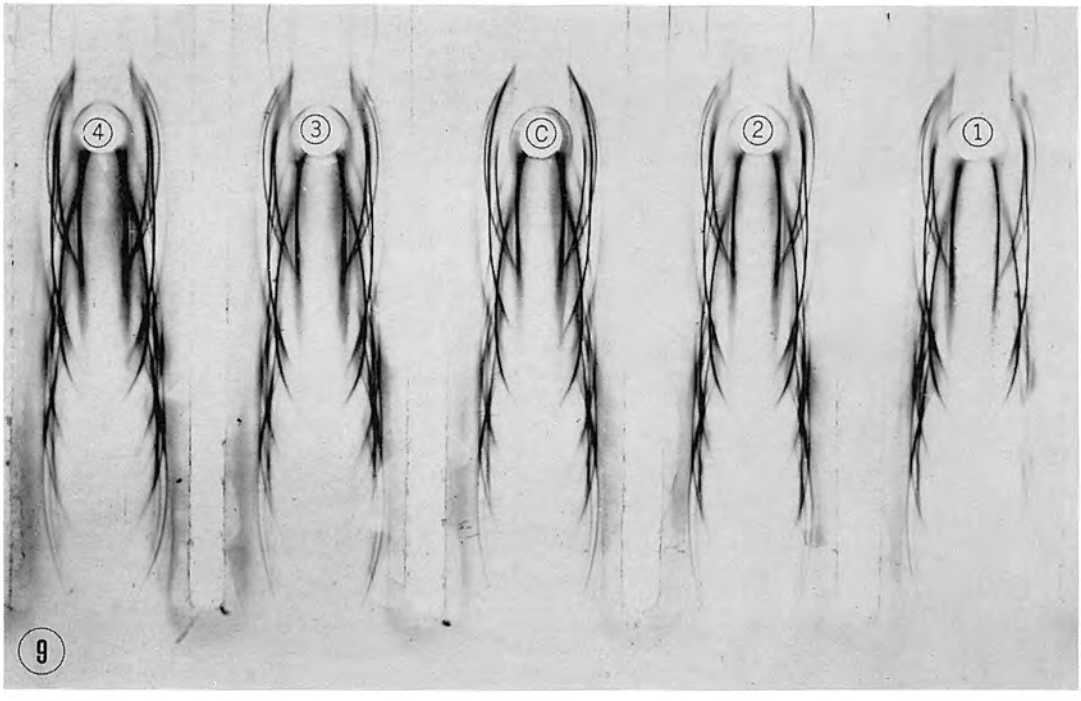
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FIGURE 10 Immunoelectrophoretic pattern of the β_{1E}-line in case 1. The β_{1E}-line was normal.
P : serum of case 1
a : horse antihuman serum
b : absorbed horse antihuman serum

FIGURE 11 Immunoelectrophoretic pattern of the β_{1E}-line in case 2. No β_{1E}-line was observed.
P : serum of case 2
a : absorbed horse antihuman serum
b : horse antihuman serum

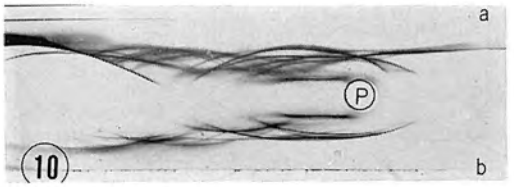
FIGURE 12 Immunoelectrophoretic pattern of the β_{1E}-line in case 2. No β_{1E}-line was ohserved.
N : normal human serum
P : serum of case 2
a and c : horse antihuman serum
b : rabbit antihuman C'4 serum

FIGURE 13 Immunoelectrophoretic pattern of the β_{1E}-line in case 3. The strength of the β_{1E}-line was slightly decreased.
N : normal human serum
P : serum of case 3
a and c : horse antihuman serum
b : rabbit antihuman C'4 serum

FIGURE 14 Immunoelectrophoretic pattern of the β_{1E}-line in case 3. The strength of the β_{1E}-line was slightly decreased.
N : normal human serum
P : serum of case 3
a and c : horse antihuman serum
b : rabbit antihuman C'4 serum

FIGURE 15 Immunoelectrophoretic pattern of the β_{1E}-line in case 4. The β_{1E}-line was very faint.
N : normal human serum
P : serum of case 4
a and c : horse antihuman serum
b : rabbit antihuman C'4 serum

FIGURE 16 Immunoelectrophoretic pattern of the β_{1E}-line in case 5. No β_{1E}-line was observed.
N : normal human serum
P : serum of case 5
a and c : horse antihuman serum
b : rabbit antihuman C'4 serum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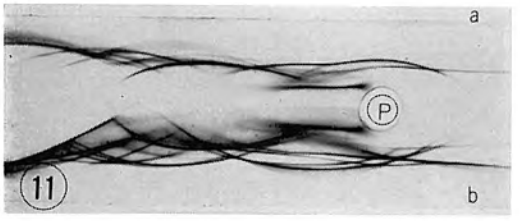
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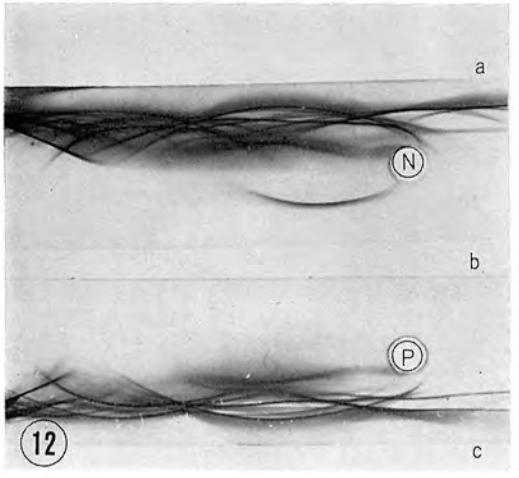
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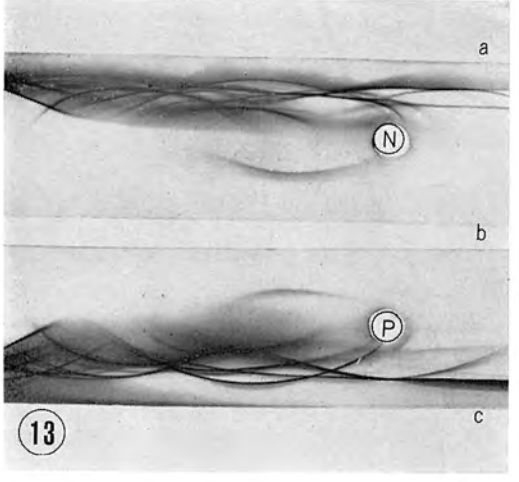
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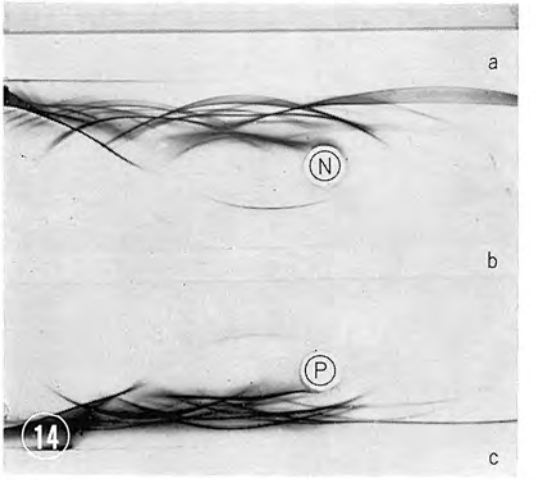
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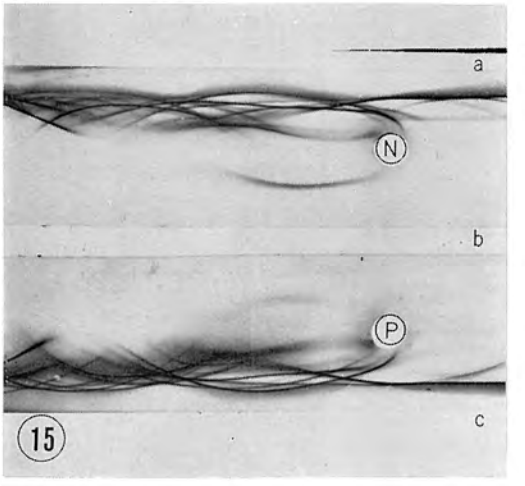
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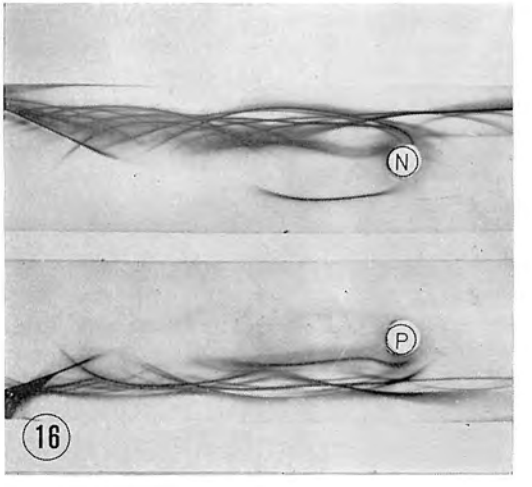
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TABLE 2 *Immunoelectrophoretic pattern of the β_{1E}-line, and the C'H50 and C'4 levels in the sera of various patients*

Case No.	Dignosis	C'H50	C'4 level ×10 eff. mol.	Appearance of β_{1E}-line	Fig. No.
1	Rheumatoid arthritis	166.0	540.0	normal	10
2	Cirrhosis of liver	3.6	not detected	not observed	11
		5.0	0.45	not observed	12
3	Cirrhosis of liver	12.5	82.5	slightly decreased	13
		15.2	not detected	slightly decreased	14
4	Cancer of stomach	10.0	not detected	hardly observed	15
5	Cirrhosis of liver	10.0	0.27	not observed	16
6	Suspected multiple myeloma	16.7	42.0	not observed	17
7	Bence-Jones type multiple myeloma	92.3	6400.0	elongated and crossed $\beta_{1C/1A}$-line	18

Case 7 (Fig. 18), which showed a persistently elevated serum C'4 level, as reported in the previous paper, exhibited an interesting immunoelectrophoretic pattern. The β_{1E}-line obtained from a sample of this patient was enlarged and crossed the $\beta_{1C/1A}$-line on the cathodal side, in parallel with the high C'4 activity. This β_{1E}-line was hardly identified and appeared to be absent in the precipitin lines formed between the patient's serum and antihuman serum. However, when rabbit antihuman C'4 serum was used, the β_{1E}-line was clearly seen. Moreover, as the horse antihuman serum used in this experiment did not contain antibody to κ-type Bence-Jones protein, rabbit antiserum against κ-type Bence-Jones protein was used for determination of the type of myeloma.

This suggests that the appearance of the β_{1E}-line is closely related with the C'4 level, but a few exceptions were seen when the β_{1E}-line was visible though the C'4 level was apparently zero. However, no case was found where C'4 was detected but no β_{1E}-line was formed.

DISCUSSION

As reported in the previous paper (INAI, *et al.*, 1967b), attempts were made to separate human C'4 from C'1 inactivator by various procedures, including DEAE cellulose column chromatography, CM-Sephadex column chromatography and Pevikon block electrophoresis.

The purified human C'4 so obtained had potent C'4 activity with a negligible amount of C'1 inactivator, and showed a single precipitin line in the β_1-globulin region by immunoelectrophoresis. It was considered that this precipitin line might correspond to the precipitin line of β_{1E}-globulin reported by MÜLLER-EBERHARD and BIRO (1963).

To see whether C'4 is identical with β_{1E}-globulin or whether C'4 activity is simply carried by molecules which are completely unrelated to, although physicochemically similar to β_{1E}-globulin, several experiments were performed by MÜLLER-EBERHARD and BIRO (1963). They concluded that β_{1E}-globulin might be identical with C'4. In this experiment, it was clearly demonstrated that the hemolytic zone of purified C'4 on an agar plate was completely limited by a single precipitin line between purified C'4 and purified

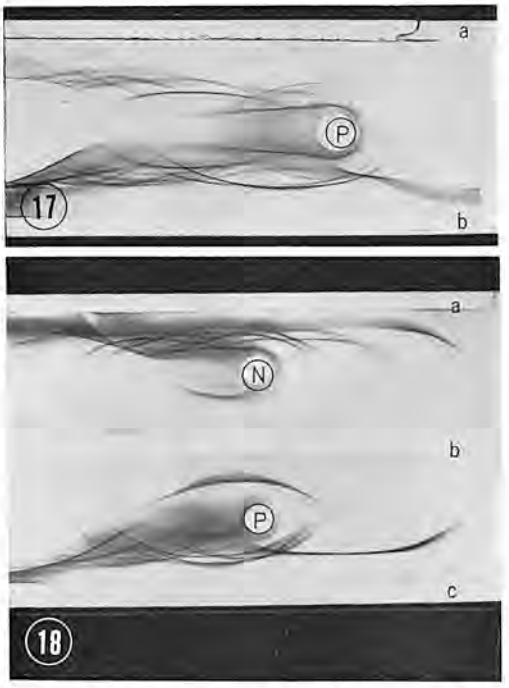

FIGURE 17 Immunoelectrophoretic pattern of the β_{1E}-line in case 6.
No β_{1E}-line or $\beta_{1C/1A}$-line was observed.
P: serum of case 6
a: absorbed horse antihuman serum
b: horse antihuman serum

FIGURE 18 Immunoelectrophoretic pattern of the β_{1E}-line in case 7.
The β_{1E}-line was elongated and crossed the $\beta_{1C/1A}$-line.
N: normal human serum
P: serum of case 7
a and c: horse antihuman serum
b: rabbit antihuman C'4 serum

horse antihuman serum on the same agar plate. The antihuman C'4 serum produced in rabbits showed a single precipitin line of β_{1E}-globulin against fresh human serum by immunoelectrophoresis. As reported in the following paper this anti C'4 serum had a remarkable capacity to neutralize the hemolytic activity of human C'4.

These results support the conclusion that human C'4 may be identical with β_{1E}-globulin.

If this conclusion is correct, the parallel between the C'4 levels in the sera of patient and the precipitin line of its β_{1E}-globulin could be explained. To investigate this problem, the β_{1E}-line in the immunoelectrophoretic pattern of fresh human serum had to be identified. There are many precipitin lines in the β_1-globulin region in the immunoelectrophoretic pattern of normal human serum, namely those of transferrin, hemopexin, β_1-glycoprotein, $\beta_{1C/1A}$-globulin, β_{1E}-globulin, fibrinogen and β_1-lipoprotein and part of both α_2-globulin and γ-globulin in this region.

To identify the β_{1E}-globulin line, immunoelectrophoresis aud Ouchterlony's double diffusion technique were employed together. To differentiate the β_{1E}-line from the $\beta_{1C/1A}$-line, goat antihuman $\beta_{1C/1A}$ serum was also used in this experiment.

Absorbed antiserum which did not contain the antibody to C'4 and $\beta_{1C/1A}$-gloublin was used to reduce the number of precipitin lines in the β_1-globulin region in the immunoelectrophoretic pattern of whole human serum. In this way, the identification of the β_{1E}-line in the immunoelectrophoretic pattern of whole human serum became easier. Thus, the β_{1E}-line runs parallel with the $\beta_{1C/1A}$-line from the antigen well to the β_1-globulin region and crossing of the two lines was never found in normal human serum.

To analyse the changes in the β_{1E}-line in the sera of patients, the changes in this line on chemical or immunological treatment of normal human serum were studied.

On treatment of purified human C'4 with hydrazine, MÜLLER-EBERHARD and BIRO (1963) reported that the electrophoretic mobility of β_{1E}-globulin increased immediately after hydrazine treatment, but the subsequently its mobility gradually decreased. PEETOOM and PONDMAN (1963) stated that treatment of normal human serum with hydrazine or immune precipitate caused a cleavage of the β_{1E}-line into two lines, β_{1E1} and β_{1E2}, using anti C' serum. In this experiment, hydrazine treatment of normal human serum resulted in the extension of the β_{1E}-line to the anodal side and crossing of the β_{1E}-line and $\beta_{1C/1A}$-line.

Treatment of normal human serum with immune precipitate also resulted in the crossing of the two lines, though C'4 activity in treated serum was moderately decreased. However no cleavage of the β_{1E}-line into two lines was observed.

From these results, it is clear that the β_{1E}-line in human serum was changed but did not disappear when the hemolytic activity of C'4 was reduced or completely destroyed by these treatment.

It was found that there was a close relationship between the C'4 level in the sera of patients and the appearance of the β_{1E}-line in their sera. In particular, scarcely any of the sera with a markedly decreased C'4 level showed a β_{1E}-line by immunoelectrophoresis. However, in a few cases, the β_{1E}-line was found, though the C'4 level was apparently zero. However, in sera with a normal or high C'4 level, absence of a β_{1E}-line has never been seen.

The discrepancies between the serum C'4 level and β_{1E}-line observed in a few cases suggest that the appearance of a β_{1E}-line in the immunoelectrophoretic pattern does not always imply the existence of C'4 activity in the sample.

From the present results it seem likely that the site of hemolytic activity of C'4 on molecules of β_{1E}-globulin might differ from that of the antigen determinant for the precipitin reaction of β_{1E}-globulin. Accordingly, the existence of β_{1E}-globulin which lacks the site of hemolytic activity of C'4 is conceivable.

ACKNOWLEDGEMENTS

The authors are indebted to Assistant Prof. Kozo Inoue, Department of Bacteriology, Osaka University Medical School for valuable advice and criticism throughout this study. They also greatefully acknowledge the technical assistance of Miss Masako Suemune.

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