BIKEN JOURNAL Vol. 10, 65-87, 1967

SERUM LEVEL OF THE FOURTH COMPONENT OF COMPLEMENT IN VARIOUS DISEASES*

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(Received February 14, 1967)

SUMMARY Decrease in the levels of complement (C′) and the fourth component of complement (C′4) were occasionally found in the serum of patients with various diseases, especially with chronic myelogenous leukemia and liver cirrhosis. Namely, decreased levels of C′ and C′4 were not found consistently in any particular diseases but were sometimes found in various diseases due to some unknown process of that disease.

A highly elevated C′4 level of about 8 times the normal C′4 level, was found in a patient who was diagnosed as having Bence Jones type myeloma.

The results failed to show any relationship between the serum C′ or C′4 level and various laboratory findings in the patients. However, laboratory findings showed that factors such as the red cell count, white cell count, platelet count, γ-globulin content in serum and icteric index definitely have no influence on the C′ and C′4 levels in the serum.

Passage of C′4 from the blood to various body fluids and its excretion in proteinuria were noted. The amount of C′4 excreted in the urine in some patients was nearly equal to the C′4 level in the serum, although the latter was maintained at the normal level. It was suggested from these results that in these patients C′4 was produced enough to make up for the excreted C′4 in the urine.

The significance of the diminution in the levels of C′ and C′4 in various diseases in which the process of immunity may participate in their pathogenesis was discussed.

* Parts of this work were reported at the 1st Symposium on Complement in August 1964 at Hakone, Japan, and at the 2nd Symposium on Complement in June at Kobe, Japan, and at the 15th Annual Meeting of the Japanese Society of Allergy in October 1965 at Tokyo.

INTRODUCTION

The relationship between serum complement levels and various diseases have been studied extensively by many investigators, and excellent reviews of these studies have been given by OSLER (1961) and LACHMANN (1962). However, only a few studies have been made on the changes of components of complement in disease (LANGE and WENK, 1954). Moreover, classical R-reagents were used by previous investigators for the estimation of components of complement in whole serum. The inadequacy of R-reagents for estimation of components has been realized, since recent studies on the separation of components of C′ and on the mechanism of immune hemolysis revealed the possible existence of eight or more components of human complement and the necessity to use cellular intermediates for the estimation of components of complement. The second component of guinea pig C′ was first estimated with cellular intermediate by BORSOS, RAPP, and MAYER (1961), and estimation of the components of guinea pig and human complement with cellular intermediates was developed by several investigators (BORSOS and RAPP, 1963, HOFFMANN, 1960, INAI *et al.*, 1964, AUSTEN and BEER, 1964). As reported in the previous paper (IANI *et al.*, 1963) diminution of the C′ level was found in a patient with chronic myelogenous leukemia, and it was found that the diminution of C′ was due to reduction of hydrazine sensitive components of complement. A remarkably low C′4 activity in this serum was confirmed by estimation of C′4 with cellular intermediates (INAI *et al.*, 1964).

The present studies were on whether diminution of C′ and C′4 activities occurs in other leukemic patients and in patients with other diseases.

This paper reports results of estimations of serum complement levels and C′4 levels in 631 samples from 296 cases with various diseases and studies on the possible roles of several factors in the changes in the C′ and C′4 levels.

MATERIALS AND METHODS

1. *Sheep erythrocytes (E), amboceptor (A) and sensitized sheep erythrocytes (EA)*

Sheep erythrocytes were preserved at 4°C with an equal volume of Alsever's solution. Before use, erythrocytes were washed twice with physiological saline and once with buffer and standardized to a concentration of 1×10^9 cells per ml.

Antibody against boiled sheep erythrocyte stromata was prepared according to the method of KABAT and MAYER (1961).

Standardized sheep erythrocyte suspension was allowed to react with an equal volume of 1 : 300 antibody for 10 minutes at 37°C.

2. *Diluents*

Isotonic saline veronal gelatin (to 0.1%) buffer of 0.15 ionic strength containing Mg^{2+} (to 5.0×10^{-4} M) and Ca^{2+} (to 1.5×10^{-4} M) is referred to as $So^{\pm}_{\pm}$. Isotonic saline sucrose veronal gelatin (to 0.1%) buffers of 0.09 ionic strength containing Mg^{2+} (to 5.0×10^{-4} M) and/or Ca^{2+} (to 1.5×10^{-4} M) are referred to as $S_4^{\pm}$ or $S_4^{\pm}$. EDTA veronal buffer was prepared by diluting 0.30 M EDTA ten fold with isotonic saline veronal gelatin (to 0.1%) buffer without added Ca^{2+} or Mg^{2+}.

3. *Materials for titration of the first and fourth components of complement*

Sera of patients and their synovial fluid, cerebrospinal fluid, ascites, pleural effusion, saliva, breast milk and urine specimen were used for titration of C′ and its components.

Almost all the leukemic patients who entered the Hiroshima Atomic Bomb Hospital and Research Institute for Nuclear Medicine and Biology, Hiroshima University from March, 1964 to August, 1965 were used in this work.

The sera of these patients were separated and frozen in ampules and transported to Osaka. Titration of C′ and its components in these sera were done as soon afterwards as possible at the Center for Adult Diseases.

About half the leukemic patients and all the patients with other diseases, who were selected at random for this work entered the hospital of the Center for Adult Diseases, Osaka.

Urine specimens were assayed on the day of collection, but other materials were used on the same day or stored at −20°C or −70°C before use.

4. *EA sensitized with the first component of guinea pig complement (EAC'1), EA sensitized with the first and fourth components of guinea pig complement (EAC'1, 4) and EA sensitized with the fourth component of guinea pig complement (EAC'4)*

These cellular intermediates were prepared as described in the previous paper (NAGAKI *et al.*, 1965).

5. *Titration of the first and fourth components of human complement*

Titration of C'1 and C'4 were done by the method described in the previous paper (NAGAKI *et al.*, 1965) with slight modifications. After 60 minutes' incubation at 37°C with C'-EDTA, all tubes received 4.5 ml of cold 0.15 M NaCl. Then the contents were mixed and centrifuged. The supernatant fluids were analysed for their oxyhemoglobin content at a wave length of 414 mμ in a Hitachi spectrophotometer using a cuvette of 1 cm light path. The normal C'4 levels of 54 human sera ranged from 3×10^{12} to 20×10^{12} eff. mol. per ml (mean, 9.91×10^{12} eff. mol. per ml).

6. *Titration of complement*

Complement was titrated as described in the previous paper (NAGAKI *et al.*, 1965). The normal complement levels of 65 human sera ranged between 70 and 110 C'H50 (mean, 84.2 C'H50). On titration of complement in normal human sera, the mean value of 1/n, the magnitude of the exponent of von Krogh's equation, was 0.210 and the standard deviation was 0.018.

RESULTS

1. *C'and C'4 levels in various diseases*

1) *Leukemia and other diseases of hematopoietic organs*

In chronic lymphocytic and myelogenous leukemia and in monocytic leukemia, C' levels have been reported as normal or elevated (BALTCH *et al.*, 1960). However, in his study, no serial estimations of C' activity or it's com-

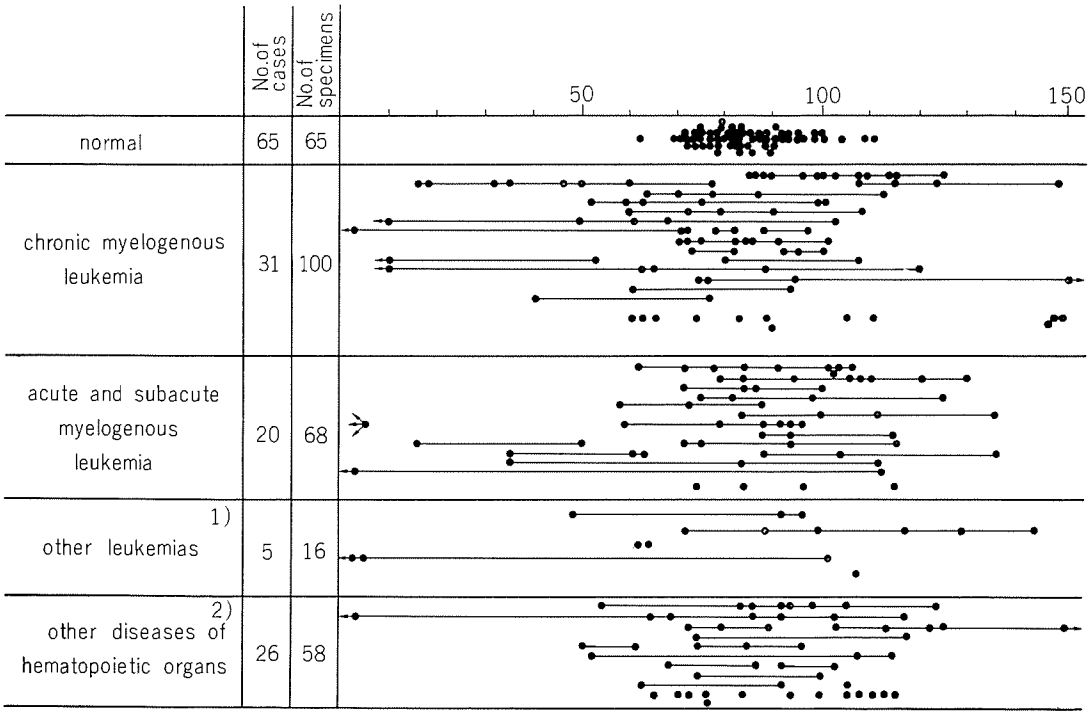

FIGURE 1a Serum C' levels of normal subjects and patients with various diseases of hematopoietic organs.

ponents were made during the courses of the diseases.

As described in the previous paper (INAI *et al.*, 1963), the C′ and C′4 levels of a patient with chronic myelogenous leukemia were successively estimated during the clinical course of the disease, and it was found that the C′ and C′4 activities fluctuated from zero to the normal range. These diminutions of C′ and C′4 levels were followed by marked increase in the white cell count. When the increase in the white cell count ceased, the C′ and C′4 activities were restored. Accordingly, the C′ and C′4 levels of the sera of the patients were estimated at intervals to see whether a similar phenomenon occurred in other leukemic patients and in patients with diseases of hematopoietic organs. The C′ and C′4 levels in 86 patients with various diseases of hematopoietic organs are summarized in Fig. 1a and 1b. The subjects includes 31 cases of chronic myelogenous leukemia, 20 cases of acute and subacute myelogenous leukemia, 5 cases of other leukemias and 26 cases of other diseases of hematopoietic

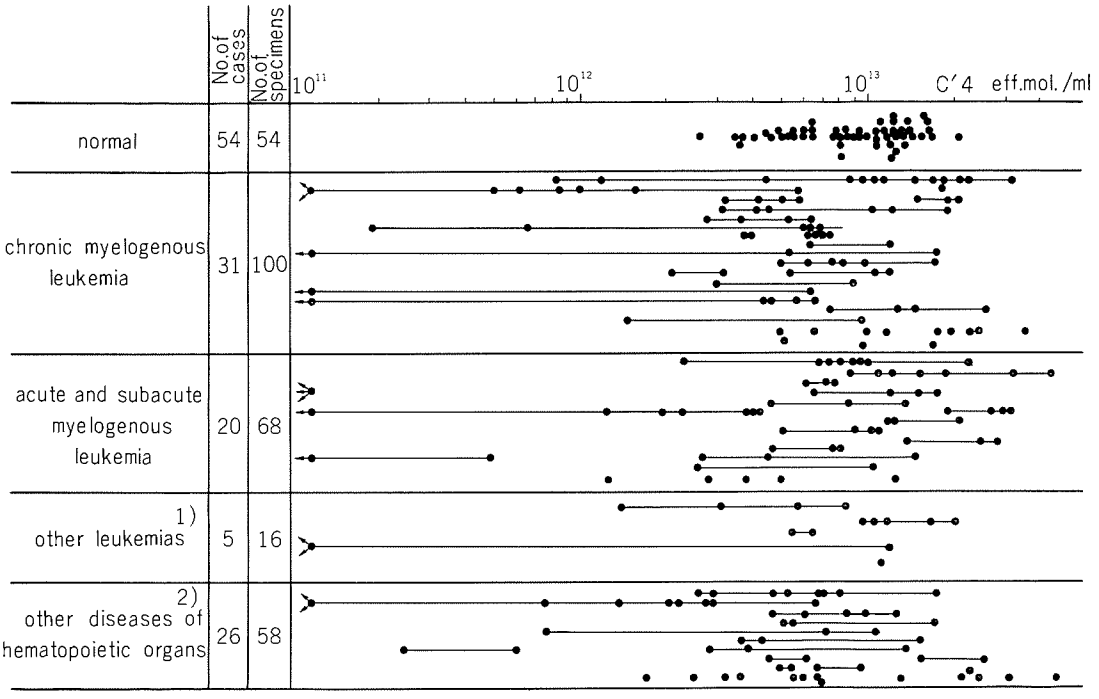

FIGURE 1b Serum C′4 levels of normal subjects and patients with various diseases of hematopoietic organs.
1) Includes 4 cases of acute lymphocytic leukemia and one case of monocytic leukemia.
2) Includes 3 cases of Banti's disease, 7 cases of aplastic anemia, 3 cases of congenital hemolytic anemia, 2 cases of polycytemia vera 2 cases of myelofibrosis and 9 cases with miscellaneous diseases of hematopoietic organs.

•——• This indicates the C′ or C′4 level of two or more serum specimens from a patient.
←•——• The arrow indicates C′ or C′4 levels below the lowest unit plotted in the figure.
The number of arrows indicates the number of serum specimens of the patient which showed C′ or C′4 levels below the lowest unit plotted in the figure.
←——• The number noted under the arrow indicates the number of serum specimens of a patient which showed
(4) C′ or C′4 levels below the lowest unit plotted in the figure.
These symbols will be used in the following Figures (8a, 8b, 13a, 13b, 17a and 17b).

organs.

Serial determinations of C′ and C′4 were carried out in 51 of these 86 patients. As shown in Fig. 1, most of the C′ and C′4 levels measured in these 86 patients were within the normal range, but wide fluctuations in the levels of C′ and C′4 were observed in several cases, especially in cases of chronic myelogenous leukemia. These fluctuations of C′ and C′4 were also observed in patients with other forms of leukemia.

Among patients with other diseases of hematopoietic organs, considerable fluctuations of both the C′ and C′4 level were found in only one case of congenital hemolytic anemia. A decrease in C′4 activity was also found in a case of myelofibrosis and in a case of hemolytic anemia, but in these cases the C′ levels were almost normal. Remarkable decreases in the hemoglobin content of the blood were found in several cases of aplastic anemia, and increased contents were found in 3 cases of polycythemia vera, but the C′ and C′4 levels of these patients were almost normal. This result shows that there is no relationship between the C′ or C′4 levels and the hemoglobin content of the blood.

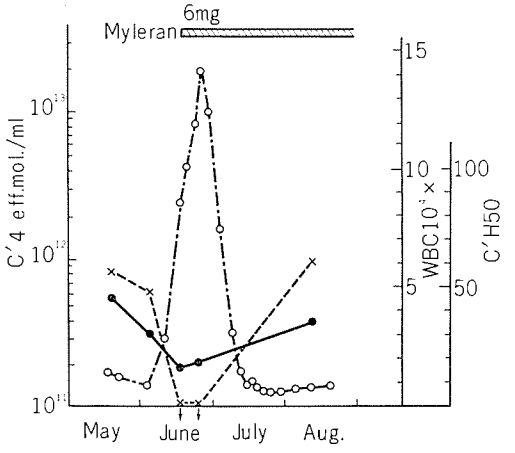

FIGURE 2 Decrease in serum C′ and C′4 levels in relation to increase in white cell count in case T.T. with chronic myelogenous leukemia.
●——● C′H50 ○·—·—○ white cell count
×— — —× C′4

Detailed findings on the clinical course of the interesting cases in this group and their C′ and C′4 levels were as follows.

T.T. chronic myelogenous leukemia

This 33-year-old male had been exposed to the atomic bomb at Nagasaki. In April, 1963 he was admitted to another hospital and diagnosed as having chronic myelogenous leukemia. In May, 1964 he was transferred to Hiroshima Atomic Bomb Hospital. At that time the spleen was palpable for 3 finger-breadths below the left costal margin.

Hematological findings: red blood cell count 430×10^4, hemoglobin 90% (Sahli), platelet count 240,800, white cell count 14,100, with 1% promyelocytes, 2% myelocytes, 78% neutrophils, 1% eosinophils, 1% basophils, 5% monocytes and 12% lymphocytes. Both the C′ and C′4 levels in his serum were moderately decreased to 46.5 C′H50 and 0.85×10^{12} eff. mol. per ml, respectively.

As illustrated in Fig. 2, after June 10 his white cell count increased gradually and reached a maximum of 143,000 on June 27. Differential count was 1.5% myeloblasts, 5% promyelocytes, 1.5% myelocytes, 3% metamyelocytes, 71.5% neutrophils, 3.5% eosinophils, 1% monocytes and 7.5% lymphocytes. The spleen enlarged gradually and was palpable for 5 finger-breadths below the left costal margin on June 27. Six mg of myleran were given daily and the white cell count decreased to 10,000 on July 12. The C′4 level on both June 16 and 24 was almost zero and the C′ levels of these specimens were very low. But on August 12 his C′4 level had increased to 0.99×10^{12} eff. mol. per ml.

He was the second case of a leukemic patient in whom the fluctuation of C′ and C′4 levels seemed to have some correlation with the white cell count. Decreases in C′ and C′4 levels were also observed in other cases with various leukemias, but these decreases were not correlated with the changes in the white cell

count.

Marked increase in the white cell count was observed in the two cases of chronic myelogenous leukemia described below, but their C'4 levels were maintained within the normal range.

M.M. chronic myelogenous leukemia

This patient was the first case observed whose C'4 levels repeatedly decreased to almost zero. She was readmitted to this hospital, because of high fever and aggravation of her hemogram. She was retreated with myleran, 6 MP, and received a blood transfusion. Clinical signs improved for a while, but soon after became worse and her white cell count increased rapidly. Myleran was stopped and prednisolone, nitromine and mytomycin were given, but on December 1, 1963 she died. As demonstrated in Fig. 3, during this last period, in spite of significant fluctuation of the white cell count, the C' and C'4 levels were not fluctuated. The most remarkable difference between the hematological findings on the last and first admissions was in the differential count, i.e., 85 to 95 per cent of myeloblasts were counted on the last admission, but on the first admission only 5 to 10 per cent of myeloblasts were found.

K.M. chronic myelogenous leukemia

This patient was diagnosed as having chronic myelogenous leukemia in 1960, and was admitted to the hospital of the Research Institute for Nuclear Medicine and Biology, Hiroshima University from 1960 to 1962. In 1963 he was readmitted to this hospital for two months. The third admission to this hospital was in May, 1965. As shown in Fig. 4, from August 9 to 23, his white cell count was within the normal range. But after this period his white cell count increased markedly and reached 185,500 on September 18. At that time, his differential count showed 47% myeloblasts,

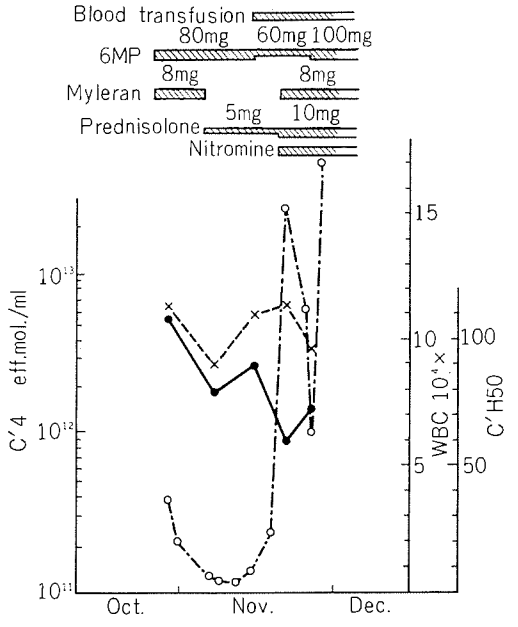

FIGURE 3 Serum C' and C'4 levels and changes in white cell count of case M.M. with chronic myelogenous leukemia in her terminal stage.
●———● C'H50 ○·—·—·○ white cell count
×— — —× C'4

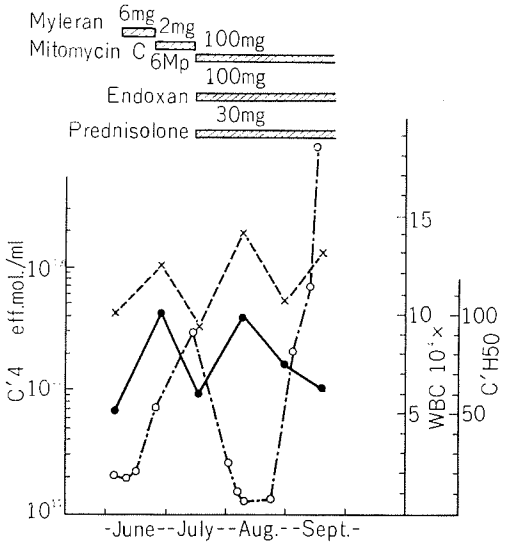

FIGURE 4 Serum C' and C'4 levels and changes in white cell count of case K.M. with chronic myelogenous leukemia in his terminal stage.
●———● C'H50 ○·—·—·○ white cell count
×— — —× C'4

3.5% promyelocytes, 0.5% myelocytes, 2% metamyelocytes, 7% neutrophils, 2% basophils, 14% lymphocytes and 24% unclassified monocyte-like cells. During this period his C′ levels were slightly reduced but no decrease in the C′4 level was recognized.

The reasons why a decrease in the C′4 level did not occur in these two cases is unknown. However, it is noteworthy that the investigations on the relationship between the changes in white cell count and serum C′4 levels were made during the terminal stages of the diseases. The increase in the proportion of myeloblasts was a remarkable common feature of the hematological findings in these two cases.

In cases of acute myelogenous leukemia, a marked increase in the white cell count was also observed, but the C′4 levels were maintained within the normal range. And in these cases, an increase in the proportion of immature leukemic cells was also recognized.

NAGAKI *et al.* (1965) reported that C′4 activity was detectable in extracts of guinea pig and human platelets, but not in extracts of leucocytes. It was also found that these C′4 activities of platelets are too small to influence the C′4 levels in the serum. This conclusion was supported by the platelet counts and C′4 levels in the next two cases.

M.N. chronic myelogenous leukemia

As shown in Fig. 5, the platelet count of this 54-year-old female was always more than 820,000 and it reached more than 2,000,000 on April 21 and remained this level for about a month. But the C′4 levels remained in the normal range during her clinical course.

Y.S. chronic myelogenous leukemia

As shown in Fig. 6, a marked thrombocytopenia was observed in a 21-year-old male with chronic myelogenous leukemia. His platelet count was less than 4,000 on March 12, 1964, and did not increase above 5,000 for two months. So serial platelet transfusions were given. Three successive measurements of his C′4 levels made over a period of two months were almost normal.

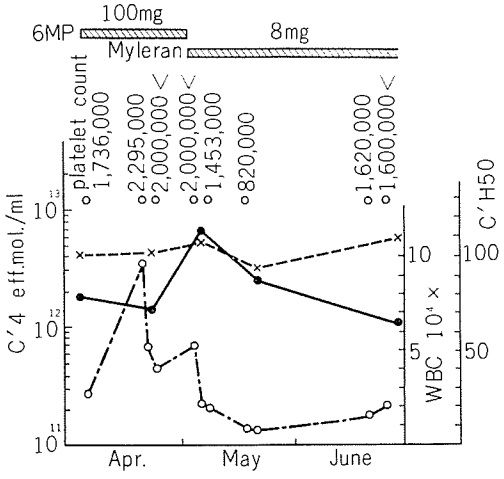

FIGURE 5 Serum C′ and C′4 levels and platelet counts of case M.N. with chronic myelogenous leukemia.
●——● C′H50 ○·—·—·○ white cell count
×— — —× C′4

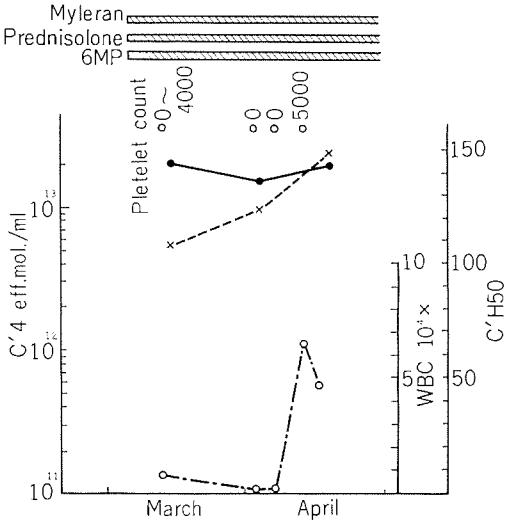

FIGURE 6 Serum C′ and C′4 levels and platelet counts of case Y.S. with chronic myelogenous leukemia.
●——● C′H50 ○·—·—·○ white cell count
×— — —× C′4

Conspicuous splenomegaly was one of the common features of the above two cases of chronic myelogenous leukemia with C′ and C′4 levels of almost zero. Accordingly, it was thought that splenomegaly might have some relation with the C′4 level in the serum. It has been reported by ROTINO (1959) that the C′ levels in patients with Hodgkin's disease, who had had splenectomy, were low or normal, but that the C′ levels of guinea pig serum, rose after splenectomy. With regard to the relation between splenectomy and the C′ level, we observed two interesting cases with splenomegaly. The one had congenital hemolytic anemia and the other Banti's disease. Their C′ and C′4 levels increased moderately after splenectomy.

As shown in Fig. 7, the C′ and C′4 levels of a 20-year-old male with Banti's disease were 52.1 C′H50 and 3.98×10^{12} eff. mol. per ml on March 16, and he was splenectomized on March 20. On April 2, his C′ and C′4 levels increased to 115.2 C′H50 and 13.9×10^{12} eff. mol. per ml, respectively. Various grades of

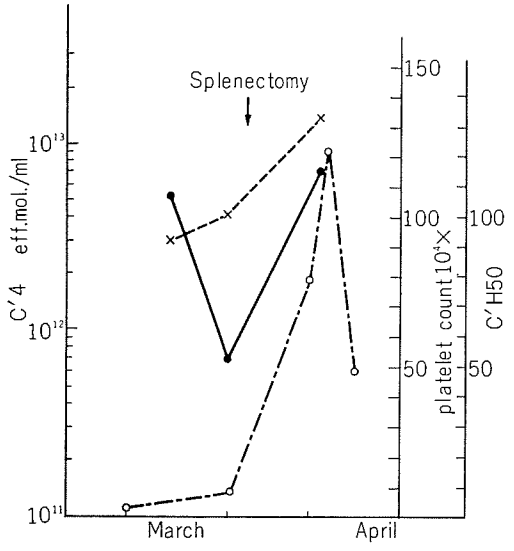

FIGURE 7 Elevation of serum C′ and C′4 levels after splenectomy in case K.H. with Banti's disease.
●———● C′H50 ○·—·—·○ platelet count
×— — —× C′4

splenomegaly were observed in patients with leukemia and other diseases, but their C′ and C′4 levels were usually within the normal range.

Therefore, the results indicate that in some patients marked splenomegaly seemed to have some influence on the serum C′ and C′4 levels, but splenomegaly may not be the cause of the decrease in the C′ and C′4 levels.

2) *Liver Diseases*

Reduction of the serum complement level in liver diseases was reported by JORDAN (1953) and MANDEL (1955). RICE (1954) succeeded in reducing the complement levels of guinea pigs by experimental liver damage. The complement and C′4 levels of patients with various liver diseases are summarized in Fig. 8a and 8b. The C′ and C′4 levels of 5 of 7 cases of acute hepatitis were within the normal range. However, both the C′ and C′4 activity in one case was slightly below the normal range, and the C′4 level in another case was moderately reduced. Among 14 cases with chronic hepatitis, the C′ levels of 5 cases and the C′4 levels of 6 cases were below the normal limit. Significant fluctuation of the C′4 level was observed in a case with lupoid hepatitis. The C′ and C′4 levels of 69 serum specimens from 30 patients with liver cirrhosis were estimated. Markedly low C′ and C′4 levels were observed in repeated estimations on three cases of liver cirrhosis. In several patients with liver cirrhosis, either the C′ or C′4 level, or both fluctuated significantly. The clinical course and C′ and C′4 levels of these patients are described below.

K.K. Liver cirrhosis and diabetes mellitus

A 57-year-old female entered the hospital because of confusion. For 3 years before admission she was treated for diabetes mellitus with BZ 55. About one year before admission she was diagnosed as having liver cirrhosis. At that time her C′ and C′4 levels were zero. Subsequently she had been treated by the family doctor, but disturbance of consciousness developed gradually for 3 months before admission to hospital. Laboratory findings

showed total protein 6.4 g/dl, 38% albumin, 62% globulin with 3% α_1-, 6% α_2-, 7% β_1-, 9% β_2- and 37% γ-globulin. Kunkel test, 26 u; thymol turbidity test, 10 u; CCF卅 in 24 hours; cobalt reaction, $R_{7(8)}$; alkaline phosphatase, 3.0 u; SGPT, 21 u; total cholesterol, 170 mg/dl; fasting glucose, 217 mg/dl; NH_3, 300 mcg/dl; urea nitrogen, 10 mg/dl

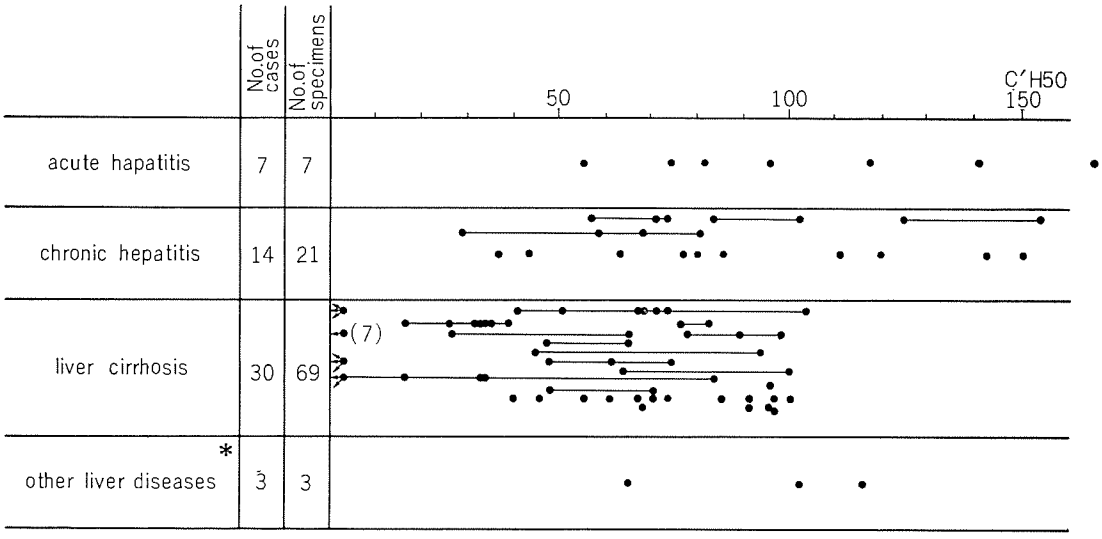

FIGURE 8a Serum C′ levels of patients with various liver diseases.

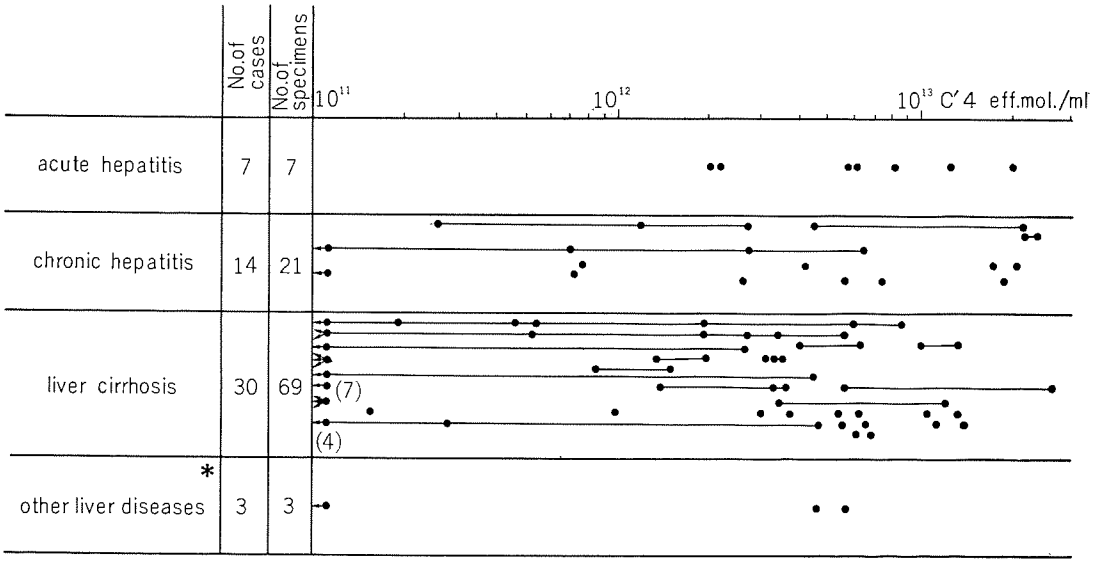

FIGURE 8b Serum C′4 levels of patients with various liver diseases.
* Includes 3 cases of cholelithiasis with icterus.

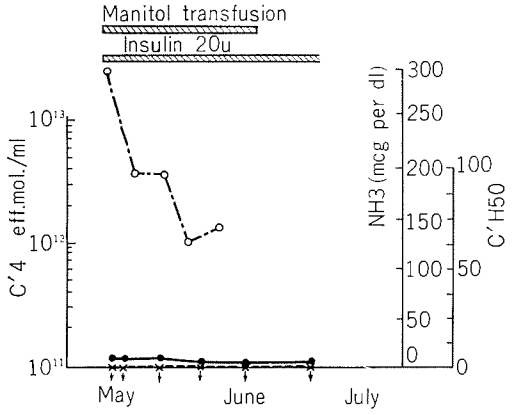

FIGURE 9 Ammonium content in blood and serum C′ and C′4 levels showing continuous low level in case K.K. with liver cirrhosis.
●———● C′H50 ○·—·—○ NH_3
×— — —× C′4

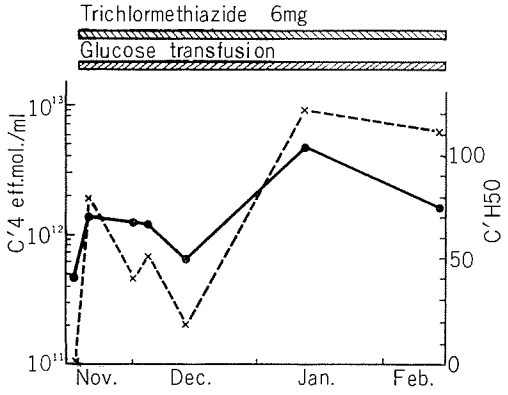

FIGURE 10 Recovery of serum C′ and C′4 levels in case Y.S. with liver cirrhosis.
●———● C′H50
×— — —× C′4

As shown in Fig. 9, fructose transfusion with several vitamines and insulin were administered daily, and after one month the blood NH_3 level decreased gradually and consciousness was recovered completely. Despite improvement of the general condition, the liver function test and serum protein pattern did not improve, and the C′ and C′4 levels also remained almost zero. Therefore, reduction of the C′4 activity in the serum of this patient may not be due to accumulation of NH_3 in the blood. Afterward her C′4 level gradually increased to 0.0066×10^{12} eff. mol. per ml on July 1, 1966, and 0.075×10^{12} eff. mol. per ml on September 2. C′ was not detectable in these samples.

Y.S. Liver cirrhosis

A 52-year-old male entered the hospital on November, 1965, because of abdominal distension and nasal bleeding. About 3 years before admission he was diagnosed as having liver cirrhosis by the family doctor and had been treated for this for a while, though later treatment was discontinued. Laboratory findings showed total protein, 7.6 g/dl with 45% albumin, 3% α_1-, 6% α_2-, 7% β_1-, 9% β_2- and 30% γ-globulin; Kunkel test, 22 u; CCF卌 in 24 hours; cobalt reaction, R_7; BSP retention, 35% after 45 min.; SGOT, 185 u; SGPT, 107 u; alkaline phosphatase, 4.1 u; and NH_3, 195 mcg/dl. Transfusion of glucose solution and vitamines, and trichlormethiazide (6 mg) was continued for one month. His physical symptoms and general condition improved and he was discharged on December 11, 1965. As shown in Fig. 10, significantly low levels of C′4 were found in determinations at intervals during his period in hospital. After discharge his C′4 level returned to normal but there was no remarkable improvement in laboratory findings.

The data on these two cases suggest that the C′4 levels of patients might return to the normal level following improvement in their general condition, but there was no direct correlation between the C′4 levels and the results of various liver function tests.

The relationship between the amount of each globulin fraction of serum and the C′ or C′4 levels was investigated, since hyperglobulinemia is generally found in chronic liver disease and in the acute stage of collagen diseases. The relation between the amount of serum γ-globulin and the C′ or C′4 level in the sera of patients with various diseases is illustrated in Fig. 11a and 11b. As shown in this figure

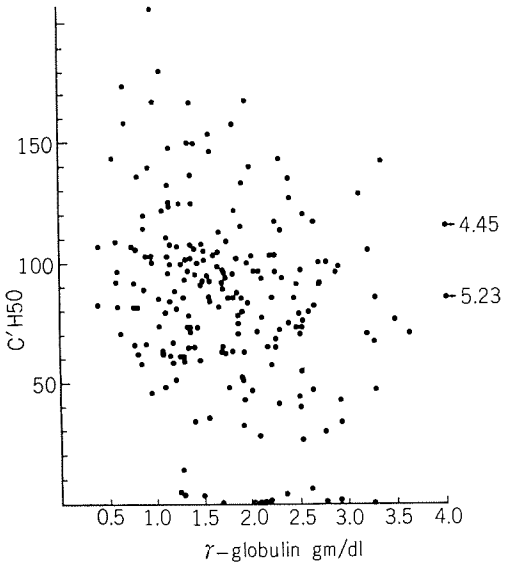

FIGURE 11a The relationship between γ-globulin and serum C′ level.

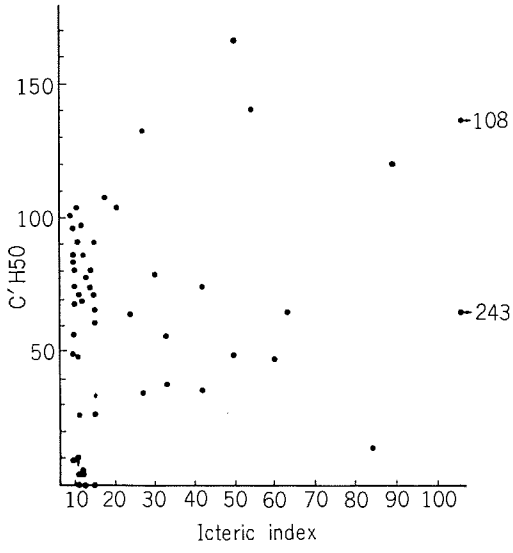

FIGURE 11b The relationship between γ-globulin and C′4 level in serum.

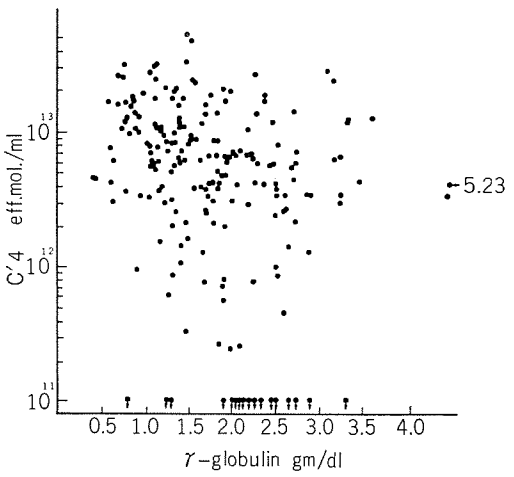

FIGURE 12a The relationship between icteric index and C′ levels.*

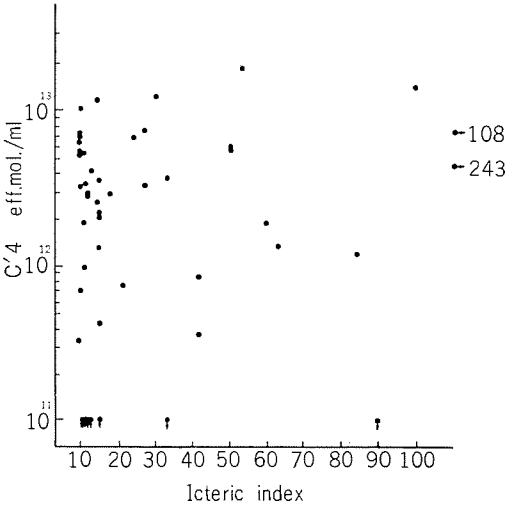

FIGURE 12b The relationship between icteric index and serum C′4 level.*

* Sera in which icteric indices are lower than 10 were excluded.

normal C′4 activities were found not only in hypo γ-globulinemia, 0.5 g/dl, but also in hyper γ-globulinemia, 4.45 g/dl. Namely, as described below, the C′ and C′4 levels in some patients with IgG-myeloma who had hyper γ-globulinemia were rather high. In patients with protein losing enteropathy or with nephrotic syndrome, with extreme hypo γ-globu-

linemia, the C′ or C′4 levels were almost normal. A number of very low C′4 levels were found in sera of patients with normal γ-globulin level.

The relationship between C′ or C′4 levels and the icteric index of the serum of patients who showed various grades of icterus is shown in Fig. 12a and 12b. It is apparent from this

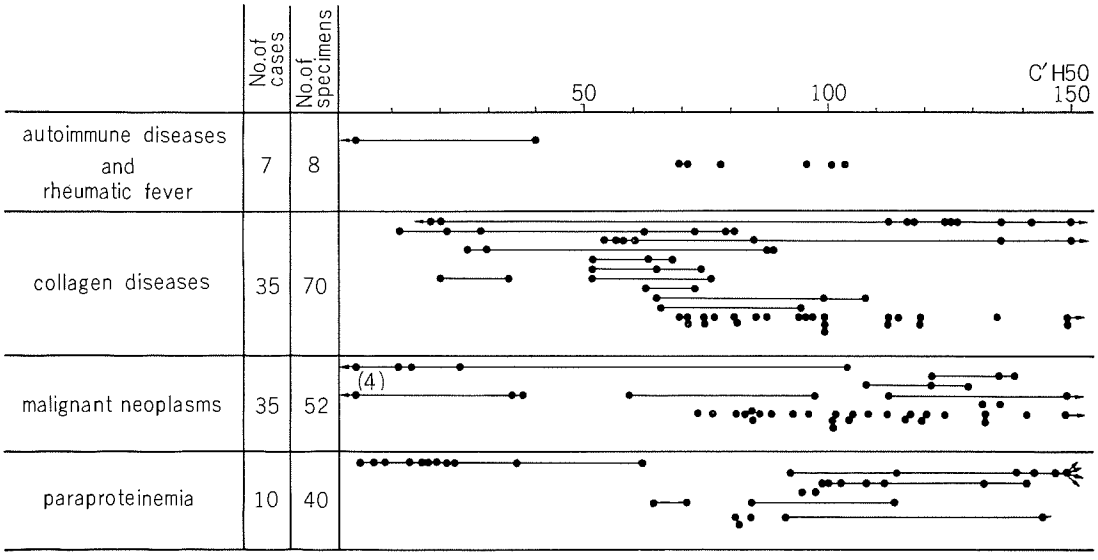

FIGURE 13a Serum C′ levels of patients with collagen disease, rheumatic fever, autoimmune disease, malignant neoplasm and paraproteinemia.

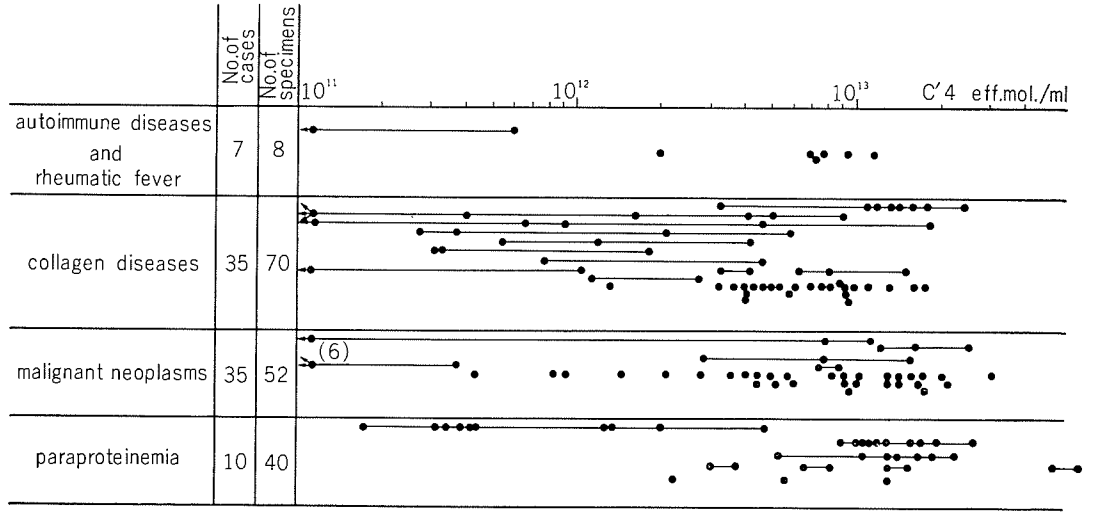

FIGURE 13b Serum C′4 levels of patients with collagen disease, rheumatic fever, autoimmune disease, malignant neoplasm and paraproteinemia.

figure that various C'4 activities were titrated in the serum of patients with a low icteric index, and C'4 activities in three serum specimens with a high icteric index of more than 90 were within the normal range. Therefore, it seems that there is no relationship between C' or C'4 and the icteric index.

3) *Autoimmune diseases, rheumatic fever and collagen diseases*

The data of patients included in this section are summarized in Fig. 3a and 3b. Four cases of autoimmune diseases including 3 cases of myasthenia gravis and one case of Hashimoto's disease were investigated.

A patient with Hashimoto's disease showed a C' level of zero and a C'4 level of 0.0135×10^{12} eff. mol. per ml. At this time, total protein was 10.4 g/dl with 46% albumin, 3% α_1-8% α_2,- 7% β_1- 8% β_2- and 28% γ-globulin. Immunoelectrophoretic analysis showed increase of three immunoglobulins. Kunkel test, 22 u; cobalt reaction, R_7; BSP retention, 17% in 45 min.; SGOT, 12 u; SGPT, 37 u; and total cholesterol, 270 mg/dl. She was not treated and her condition remained unchanged for a while. But four days later, the C' and C'4 levels rose to 40.3 C'H50 and 0.6×10^{12} eff. mol. per ml, respectively. The course of this patient was not followed. The C' and C'4 levels of other patients in this group were within the normal range.

The serum C' and C'4 levels of three cases with rheumatic fever were almost all within the normal range.

The C' and C'4 levels of 35 patients with collagen disease including rheumatoid arthritis, systemic lupus erythematosus (S.L.E.), periarteritis nodosa, scleroderma, primary chronic diffuse pulmonary fibrosis, etc. varied widely. The course of an interesting case of systemic lupus erythematosus, who showed significant fluctuation of the C'4 level, is described below.

A.Y. Systemic lupus erythematosus

This patient, a 35-year-old female, was admitted to the hospital on September 6, 1964

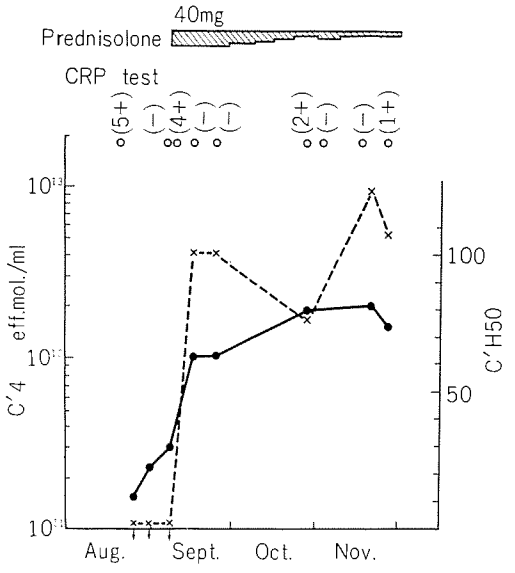

FIGURE 14 Fluctuation of serum C' and C'4 levels and of CRP in case A.Y. with systemic lupus erythematosus.
●——● C'H50 ×— — —× C'4

because of fever of 2 months' duration, facial erythema, polyserositis, multiple joint pains and albuminuria. She was diagnosed as having S.L.E., though repeated L-E cell preparations and L-E tests were negative. Her clinical data were CRP 5+; RA±; total protein, 6.1 g/dl with 32% albumin, 6% α_1,- 12% α_2-12% β_1,-4% β_2-and 34% γ-globulin. The ESR was 64 mm in one hour. As illustrated in Fig. 14, at the time of admission, her C' and C'4 levels were very low. A dose of 40 mg of prednisolone was given daily. The condition of the patient gradually improved together with increment of C' and C'4 levels. On October 27, the dose of prednisolone was reduced to 10 mg. Two days later subfever developed with a positive CRP and acceleration of the ESR. The C'4 level decreased again. But when the dose of prednisolone was increased to 15 mg per day, the patient's condition improved with a negative CRP and the C'4 level increased.

But one case with Wegener's granulomatosis showed significant fluctuation of the C′ and C′4 levels which were not related to the patient's condition.

4) *Neoplasms*

Most of the 35 patients examined with malignant neoplasms had normal C′ and C′4 levels. Only two cases showed significant fluctuation of C′ and C′4 levels. Details of one of these two cases are described below.

K.N. Stomach cancer

As shown in Fig. 15, a 58-year-old male was admitted to this hospital because of stomach cancer on December 19, 1965. Laboratory findings at that time were normal. But the C′ and C′4 levels of his serum, which were determined three times before operation, were markedly low. On January 19, 1966, gastrectomy was performed, and he was transfused with 2400 ml of whole blood during and after the operation. On January 26, the C′ and C′4 levels returned to normal, but both levels soon decreased again and remained at a low level thereafter. The only abnormal laboratory findings after operation were slight increases in β_2- and γ-globulin in the serum. The C′1 level

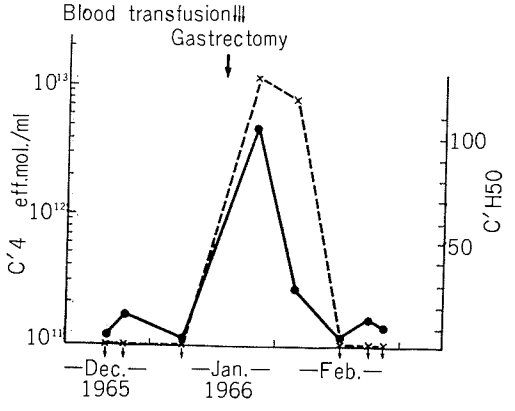

FIGURE 15 Recovery of serum C′ and C′4 levels after gastrectomy in case K.N. with gastric cancer.
●——● C′H50 ×— — —× C′4

of his serum was within the normal range throughout.

The C′ and C′4 levels of a case with liver cancer and possible liver cirrhosis were estimated 3 times in July 1965. The C′ levels in his sera were always below the normal range. Marked diminution of the C′4 level was also observed in these sera. Liver cirrhosis should be taken into consideration more than liver cancer as a cause of diminution of C′ and C′4 levels in this patient.

5) *Paraproteinemia*

In this group, 7 cases of IgG-myeloma, one case of Bence Jones type myeloma, one case of macroglobulinemia and one case of hyperglobulinemia of unknown etiology are included.

As indicated in Fig. 13, one case of IgG-myeloma and one case of hyperglobulinemia showed high C′ and C′4 levels. A surprisingly high level of serum C′4 was found in the patient who was diagnosed as having Bence Jones type myeloma.

The C′4 level of this patient was estimated twice in January, 1967, and found to be 64.0 and 52.5×10^{12} eff. mol. per ml, respectively. Studies on the clinical course and the changes in C′4 level of this patient are being followed. However, a possible case of IgG-myeloma showed low C′ and C′4 levels. Details of the clinical course and C′4 levels of this patient were as follows.

T.M. Possible myeloma

This 59-year-old male was admitted to the hospital because of vague lumbago, generalized edema and hypertension on February 18, 1966. Laboratory findings at the time of admission showed ESR, 122 mm in one hour; hemoglobin, 9.5 g/dl; hematocrit, 29%; white cell count, 6600; platelets, 208,000; and total protein 5.3 g/dl with 37% albumin, 5% α_1-, 13% α_2-, 11% β_1-, 6% β_2- and 28% *monoclonal* γ-globulin. Serum immunoelectrophoresis revealed marked increase of IgG and decrease of IgA and IgM. Analysis of urine

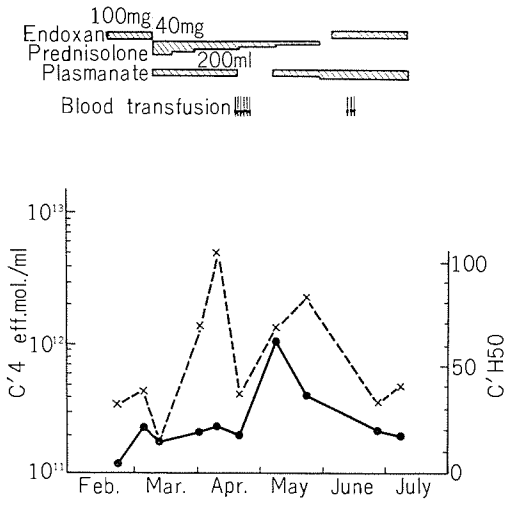

FIGURE 16 Fluctuation of C′ and C′4 levels and the course of case T.M. with possible myeloma.
●——● C′H50 ×— — —× C′4

showed ## albumin. Microscopical analysis showed numerous red cells and a few hyaline and granular casts in a high power field. The test for Bence Jones protein was negative. Repeated bone marrow aspirations failed to demonstrate myeloma cells. Bone X-ray examination revealed generalized osteoporosis, but no punched out lesions were seen.

As illustrated in Fig. 16, he was given endoxan, 100 mg, every day, but becasue of nausea and poor appetite, this was replaced by prednisolone on March 10. The C′ and C′4 levels were markedly low during this period. At the end of March, streptococcal septicemia and pyothorax developed accompanied with fever, chest pain and multiple joint pains. These were completely cured with antibiotics almost a month later. During the active period of sepsis, the C′4 level rose to normal, but fell to the initial level at the time of recovery from sepsis. At the end of April, 2,000 ml of blood were transfused, and this was followed by increase of the C′ and C′4 levels to the lower limit of the normal range. This increase of C′ and C′4 levels was transient and the levels gradually dropped to the initial values, and these did not respond to the second transfusion of 1,600 ml of blood in the middle of June. He died on July 19.

6) *Kidney Diseases*

The complement level in kidney diseases has been thoroughly investigated by LANGE *et*

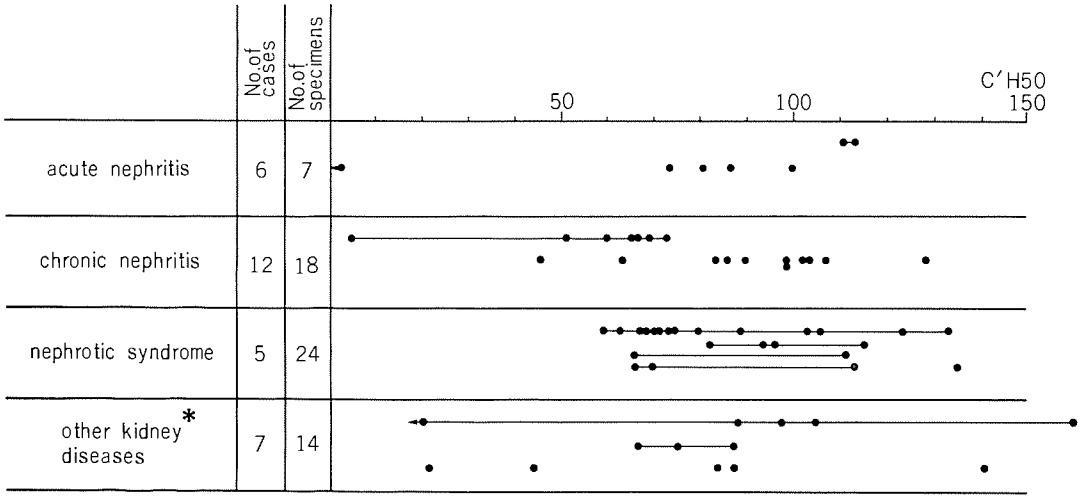

FIGURE 17a Serum C′ levels of patients with various kidney diseases.

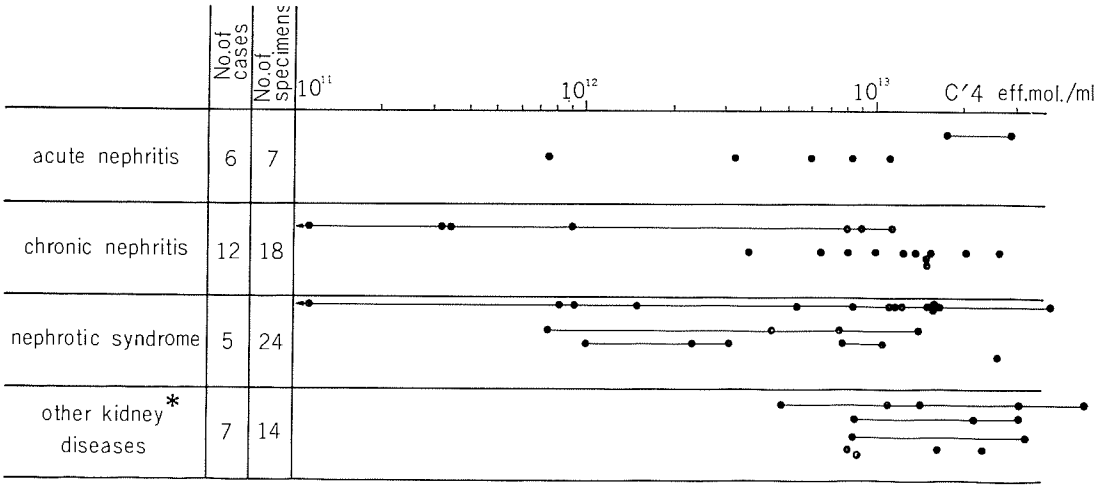

FIGURE 17b Serum C'4 levels of patients with various kidney diseases.
* Includes 3 cases of Kimmelstiel-Wilson's disease, 2 cases of uremia from nephrosclerosis, one case of movable kidney and one case of kidney tumor with hydronephrosis.

al. (1960). They stated that C' levels were low in acute glomerulonephritis and in nephrosis. Moreover, they reported that in some cases of the nephrotic syndrome reduction of C'4 activity in the serum could be demonstrated with R-reagent. In the present work the C' and C'4 activities of 63 serum specimens from 30 cases with various kidney diseases were estimated. The results are shown in Fig. 17a, b. Among 6 cases of acute glomerulonephritis, reduction of C' and C'4 levels was found only in one case. The C' and C'4 levels in a case with glomerulonephritis were estimated twice successively soon after the onset of her disease. Both activities were within the normal range. The reason why no reduction of C' was observed is not clear, but might be because the symptoms were mild. In chronic glomerulonephritis all cases had normal C' and C'4 levels except one case in whom both activities fluctuated significantly. Details of the fluctuation of the C' and C'4 levels and the clinical course of this case are described below, and shown in Fig. 18.

Among 5 cases of the nephrotic syndrome 3 cases showed low C' and C'4 levels more than once during their course, but the other cases showed normal levels. Detailed results of an interesting case with a nephrotic syndrome whose C' and C'4 levels fluctuated significantly are described below and shown in Fig. 19.

In other kidney diseases, including 4 cases of Kimmelstiel-Wilson syndrome, the serum C'4 levels were normal but low C' levels were estimated in some cases.

LANGE and WENK (1954) reported excretion of C'4 in the urine in the nephrotic syndrome as determined with R-reagent. It is possible that excretion of some components of complement may cause reduction of its components and complement activity in the serum. Therefore, the activities of C' and C'4 in the urine of normal persons and of patients with various diseases were estimated and the results are described in the following section. The effect of excretion of C'4 in the urine on the C' or C'4 levels in the serum was studied as shown in the following case reports.

S.K. Chronic glomerulonephritis

Twenty-four years prior to admission, this 54-year-old male had been treated for 3 months for acute nephritis. For 6 months before admission he had been treated by a physician

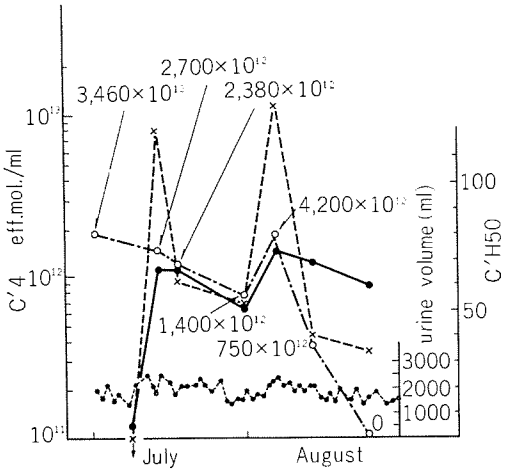

FIGURE 18 Fluctuation of serum C′ and C′4 levels and of daily amount of C′4 excreted in the urine in case S.K. with chronic glomerulonephritis.
●———● serum C′H50
×– – –× serum C′4
○—·—·○ C′4 in urine (eff. mol. per ml)
●------● urine volume
The numbers in the figure indicate total amount of urinary C′4 excreted per day.

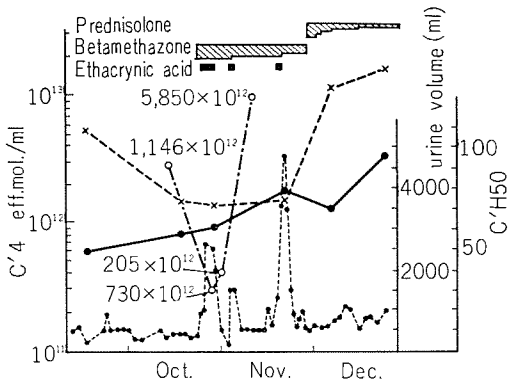

FIGURE 19 Serum C′ and C′4 levels and daily amount of urinary C′4 excreted in case M.O. with nephrosis.
●———● serum C′H50
×– – –× serum C′4
○—·—·○ C′4 in urine (eff. mol. per ml)
●------● urine volume
The numbers in the figure indicate the total amount of urinary C′4 excreted per day.

because of edema and proteinuria, but as no improvement was seen, he was admitted to the hospital on June 16, 1965. On admission his laboratory findings were as follows; the urine gave a ## test for protein, + test for sugar and the sediment contained 30 red cells, 7 to 8 white cells and a few hyaline casts per high power field. Total protein was 4.4 g/dl; A/G, 0.69; cobalt reaction, Ro; total cholesterol, 316 mg/dl; fasting glucose, 80 mg/dl; urea nitrogen, 21 mg/dl; Ca, 7.1 mg/dl; Na, 142 mEq; K, 4.2 mEq and Cl, 115 mEq per l. Blood examination revealed that the red cell count was 298×10^4, hemoglobin, 9.5 g/dl; color index, 1.0; hematocrit, 29%; white cell count, 8,100 with normal differential; RPF, 142 ml; GFR, 19 ml/min; F.F., 0.14; PSP excretion in 15 min., 7%. One hundred ml of plasmanate was given daily, with a low salt diet. The C′4 levels in his serum and in urine were estimated seven times. On July 13 and August 6 the C′4 levels in the serum were within the normal range, but the C′4 levels in other serum specimens were low.

There was no relationship between these fluctuations in the C′4 level and his clinical condition. The fluctuation of the C′4 level in the serum and urine, and daily urine volume are shown in Fig. 18. The daily urine volume was relatively unchanged, and a remarkable amount of C′4 was excreted continuously in the urine. A parallelism between the fluctuation in the C′4 level in the urine and that in the serum was also observed. It is apparent that the reduction of the C′4 level in the serum might not be caused by excretion of C′4 in the urine, because despite increase of C′4 excretion in the urine its level in the serum was elevated.

M.O. Nephrotic syndrome

This 16-year-old girl was referred to us from another hostpital on April 24, 1964. One year before admission she had noticed edema of the lower extremities. This sign gradually increased and in October 1963 she entered the other hospital and was treated with several

corticosteroids and diuretica. Her nephrotic signs were not improved and Cushing's syndrome developed. Laboratory findings on admission are described below; serum total protein 4.7 g/dl, with 24% albumin, 6% α_1-, 30% α_2-, 15% β-$_1$, 7% β_2- and 18% γ-globuling; total cholesterol, 666 mg/dl; urine, ## protein; + sugar; and 0 to 1 red cell, and a few hyaline, and transitional cell casts per high-power field. During stay in hospital the serum C′ and C′4 levels were measured at intervals and the C′4 level in the urine was also measured occasionally. Fig. 19 shows the C′ and C′4 levels in the serum, the urinary C′4 levels and the daily urine volumes from September 16 to December 8, 1964. During this period her serum C′4 levels were below the normal limit on October 19 and October 27, 1964. The urinary C′4 level was 2.92×10^{11} eff. mol. per ml on October 19. At that time edema increased and betamethazone and diuretica were given. Thereafter, the daily urine volume increased and edema decreased. On November 10, the C′4 level in the urine increased to 9.0×10^{12} eff. mol. per ml and the total amount of C′4 excreted into the urine reached $5{,}850\times10^{12}$ eff. mol. per day.

Thus, in this patient considerable C′4 were excreted everyday in the urine. Moreover, in November the amount of C′4 excreted in the urine was markedly increased but the serum C′4 level was also slightly increased.

The relationship between the C′4 level in the serum and the amount of C′4 excreted in the urine, found in these two patients, suggests that the production of C′4 might be sufficient to make up for the daily loss of C′4 in the urine.

2. *Complement Components in Urine and Body Fluids*

As mentioned above fluctuations in the serum C′ and C′4 levels were found in some patients with various diseases, but no correlation was found between the result of laboratory tests on these patients and the changes in the serum C′ and C′4 levels. It is quite possible that passage of C′ and its components from circulating blood into other tissues and the urine may influence the levels of serum C′ or its components very much. Therefore, the activities of C′ and its components in urine and various body fluids were estimated.

1) *Urine*

As mentioned above, C′4 was excreted in the urine of patients with various kidney diseases. Accordingly, the C′, C′1 and C′4 activities and the protein content of the urine of patients who showed various degrees of proteinuria were estimated. Urine specimens were centrifuged at 3,000 rpm for 15 minutes, and the supernantants were used for these estimations. The protein content was determined by Sueyoshi's method. Urine from 4 healthy subjects in this laboratory, whose urine did not give any protein reaction, were also investigated. Traces of C′4 were detected in these normal samples, but the actual amounts were too small to measure.

Table 1 shows that the urine of a patient with nephrosis, M.O., contained 9.75×10^{12} eff. mol. per ml of C′4 and in other urine samples significant amounts of C′4 were also found, but the C′4 activities were not always parallel with the protein contents. On the other hand, C′1 activity could only be detected in the urine of one patient with myeloma and not in other samples. No C′ activity was found in any of the samples examined.

It is very interesting that although C′4 is usually excreted C′1 is not. However, the possibility, that subcomponents of C′1 are excreted in the urine, can not be neglected. Hemolytically active C′1 may not be excreted in the urine because it is a macromolecule.

2) *Ascitic fluid*

The C′, C′1, and C′4 levels of ascitic fluid from 3 cases with liver cirrhosis, 5 cases with peritonitis carcinomatosa, and one case with a pelvic abscess were measured. The cells in

TABLE 1 *Components of complement in urine*

Case	Diagnosis	C'1/ml eff. mol. $\times 10^{-10}$	C'4/ml eff. mol. $\times 10^{-10}$	Protein (g/dl)
S. I.	normal	0	trace	—
S. Y.	normal	0	trace	—
K. F.	normal	0	trace	—
K. N.	normal	0	trace	—
M. O.	nephrosis	0	27.4	0.15
M. O.	nephrosis	0	975.0	2.4
K. E.	nephrosis	0	86.0	0.9
K. S.	nephrosis	0	215.0	6.0
J. O.	subacute glomerulonephritis	0	0.51	0.15
Y. M.	chronic glomerulonephritis	0	0.435	0.20
S. M.	chronic glomerulonephritis	0	5.40	0.60
S. K.	chronic glomerulonephritis	0	192.0	0.85
S. K.	chronic glomerulonephritis	0	78.0	0.5
H. K.	Kimmelstiel-Wilson's syndrome	0	96.0	0.8
I. B.	IgG-myeloma	0.75	3.70	0.8

TABLE 2 *Complement and its components in ascitic fluid*

Case	Diagnosis	C'H50	C'1/ml eff. mol. $\times 10^{-10}$	C'4/ml eff. mol. $\times 10^{-10}$	Protein (g/dl)
K. Y.	liver cirrhosis	0	125.0	3.76	1.6
S. N.	liver cirrhosis	7.5	141.0	24.4	1.0
T. Y.	liver cirrhosis	4.42	90.0	11.8	1.7
M. T.	peritonitis carcinomatosa	40.0	108.0	950.0	3.8
K. O.	peritonitis carcinomatosa	38.5	139.0	213.0	4.2
K. B.	peritonitis carcinomatosa	41.2	50.5	285.0	—
S. O.	peritonitis carcinomatosa	52.5	157.0	312.0	4.0
H. T.	peritonitis carcinomatosa	35.8	97.0	94.5	4.4
Y. H.	pelvic abscess	54.1	133.0	412.0	6.9

TABLE 3 *Complement and its components in various body fluids*

Case	Materials	Diagnosis	C'H50	C'1/ml eff. mol. $\times 10^{-10}$	C'4/ml eff. mol. $\times 10^{-10}$	Protein (g/dl)
M. O.	pleural fluid	tuberculous pleurisy	58.7	41.6	110.0	4.6
S. M.	pleural fluid	pleuropericarditis	25.7	114.0	184.0	9.0
R. N.	pleural fluid	postoperative pleurisy	45.0	94.5	135.0	—
Y. M.	pleural fluid	carcinomatous pleurisy	62.5	112.5	780.0	—
S. K.	pleural fluid	postoperative pleurisy	30.8	86.0	237.0	3.3
I. T.	pleural fluid	pyothorax	0	0	0	2.6
K. T.	pleural fluid	unknown etiology	51.0	421.0	214.0	4.4
I. K.	synovial fluid	rheumatoid arthritis	34.1	313.0	158.0	3.6
K. N.	saliva	normal	0	—	0	—
K. F.	saliva	normal	0	—	0	—
O. A.	breast milk	normal	0	0.02	14.2	—

the ascitic fluid were centrifuged off, and the supernatants were used for the estimation. The protein contents of these supernatants were determined with a Hitachi protein refractometer. As shown in Table 2, in the samples from patients with liver cirrhosis both the C′ and C′4 level were lower than those from other patients. The difference between the activities of complement and C′4 in ascites from patients with liver cirrhosis and those with other diseases seems to be due to the low protein content of the ascitic fluid of the former. However, the C′1 activity in ascites was not correlated with the protein content.

3) *Other body fluids*

Pleural effusions from patients with various diseases were obtained by pleural puncture, centrifuged at 3,000 rpm for 15 minutes and the supernatants were used. The protein contents of these supernatants were determined with a Hitachi protein refractometer. As shown in Table 3, in 6 of the 7 specimens of pleural exudate examined, there were significant amounts of C′, C′1 and C′4. However, in one specimen from a case of pyothorax no C′, C′1 or C′4 activity could be detected. In this case, components of complement in the putrefied fluid might have been destroyed by proteolytic enzymes.

In a specimen of fluid from a joint of a patient with rheumatoid arthritis, significant amounts of C′, C′1 and C′4 were found, and the protein content was 3.6 g/dl. No C′ or C′4 activity was found in two specimens of saliva from normal subjects.

The existence of C′4 in human milk was suggested by PEETOOM and PONDMAN (1964) from immunoelectrophoretic studies. Human breast milk was centrifuged at 10,000 rpm for 15 minutes and the supernatant was used for titration of C′, C′1 and C′4. A trace of C′1 and low C′4 activity was found. But no total C′ activity could be detected in this milk.

Nine specimens of spinal fluid from various patients were examined. As demonstrated in Table 4, small amounts of C′1 and C′4 were found in every case, but no C′ activity was found.

TABLE 4 *Complement and its components in spinal fluid*

Case	C′H50	C′1/ml eff. mol. $\times 10^{-10}$	C′4/ml eff. mol. $\times 10^{-10}$
T. S.	0	5.07	0.95
S. Z.	0	1.25	1.87
T. N.	0	3.58	0.2>
H. M.	0	1.95	3.90
O. A.	0	2.97	0.54
N. T.	0	2.34	1.18
T. O.	0	0.74	0.31
B. Z.	0	4.12	0.75
Y. O.	0	1.01	6.07

DISCUSSION

It has been shown that cellular intermediates must be employed for estimation of complement components. Using this method determination of complement components in the serum of patients may offer valuable information about the relationship between the levels of serum complement and its components and conditions in various diseases, especially those diseases in which the pathogenesis is influenced by the immunological process. However, there is little information available on the factors which influence the changes in the levels of complement and its components in various diseases, i.e., production of complement components, consumption of components by immunological processes *in vivo*, destruction of components by unknown mechanisms and their excretion and passage from peripheral blood, etc.

This work was to find other cases showing significant fluctuations in the levels of C′ and C′4 like in the first case of chronic myelogenous leukemia encountered, and to clarify the factors which influence the serum C′ and C′4 levels.

Only one of the many cases of chronic myelogenous leukemia studied showed significant fluctuations in the C′ and C′4 levels like the first case of chronic myelogenous leukemia. In

these two cases, decrease in the levels of C′ and C′4 was followed by a marked increase in the white cell count. However, the number of myeloblasts did not increase. As described in the results, in two other cases of chronic myelogenous leukemia marked increase in white cell counts were also observed in the terminal stages of the disease, but no decrease in the C′ and C′4 levels was demonstrated. The increase in the proportion of myeloblasts was a conspicuous common feature of these two cases. However, several patients with other diseases showed fluctuation and significant diminution of C′ and C′4 levels, especially in cases of systemic lupus erythematosus, liver cirrhosis and other leukemias. From these results, it is obvious that a decrease in the C′ and C′4 levels is not a characteristic of any specific disease, but is related to some unknown clinical conditions in various diseases. To investigate possible factors which might influence the changes in the C′ and C′4 levels, the correlations between laboratory findings and the C′ and C′4 levels were thoroughly studied. The present work shows that the C′ and C′4 levels in serum were not influenced by changes in laboratory results such as the red blood cell count, white blood cell count, platelet count, several liver function tests, serum protein concentration, and especially the amount of γ-globulin, the icteric index, etc.

Elevated C′ levels following splenectomy were reported by JORDAN (1953), and elevated C′ and C′4 levels following splenectomy were also demonstrated in sera of two patients in this study. These observations and the fact that marked splenomegaly was observed in two cases of chronic myelogenous leukemia who showed typical fluctuations in the C′ and C′4 levels suggest the possible role of the spleen in the changes in the C′ and C′4 levels in the serum.

Few cases with elevated C′4 levels were found, but a cases of Bence Jones type myeloma showed a C′4 level, which was about 8 times the normal level.

THORBECKE *et al.* (1965) studied the sites of syntheses of complement components by autoradiography, but the exact sites are not known. But as with other serum proteins, the liver may be important in production of C′4, because decreased C′ and C′4 levels were found in several cases of liver diseases.

With regard to diminution of C′ and C′4 levels, participation of immune reactions in the pathogenesis of such diseases as systemic lupus erythematosus, Hashimoto's disease, myasthenia gravis and probably liver cirrhosis and nephritis should be considered. However, it seems unlikely that the diminution of C′ and C′4 levels in these diseases can be attributed merely to the immune process, because despite marked decreases in the C′ and C′4 levels in some patients with these diseases, the C′1 levels were almost always maintained within the normal range. The relationship between the levels of C′1 and C′4 in sera with a low C′ titer is described in the next paper. Moreover, some patients with kidney diseases excreted large amounts of C′4 in the urine everyday, but the C′4 levels in their sera remained unchanged. Excretion of some complement components in the urine was observed by LANGE and WENK (1954) and by LANGE *et al.* (1960), but they emphasized that the amounts of the components excreted in the urine were too small to explain the low C′ titers in the sera. As seen from the present results, the amounts of C′4 excreted in the urine in cases of severe proteinuria were not so small as those reported by LANGE *et al.*, and in these patients the serum C′4 levels were maintained within the normal range. Loss of C′4 from the serum in the urine may be counteracted by its new synthesis. These facts suggest the possible existence of a mechanism regulating C′4 production and this may maintain the C′4 level in the serum when large amounts of C′4 are lost by excretion or are consumed.

Thus, as causes of decrease in the C′ and C′4 levels in sera of patients with diseases where immune processes are involved, not only consumption of C′4 by the antigen-antibody reaction but also disorders of production or syn-

thesis of C'4 should be taken into consideration.

ACKNOWLEDGEMENT

The authors wish to acknolwedge the valuable advice and criticism throughout this study of Dr. Nobuyuki Senda, Associate Director of the Center for Adult Diseases, Osaka and of Prof. Tsunehisa Amano, Department of Bacteriology, Osaka University Medical School. We are also indebted to Dr. Shozo Inoue of Hiroshima Red Cross Hospital, for allowing us to study some of his cases, and to Miss Toshiko Yoshiba and Miss Taeko Kaizu for their skilled technical assistance.

ADDENDUM

As described in materials and methods, the mean value of 1/n of normal human sera was 0.210 and the standard deviation was 0.018. But in some patients with various diseases, hardly measurable hemolysis was seen even when 2.0 ml of 10 times diluted serum were used. In such cases, C'H50 values were recorded as below 5 C'H50, and it was impossible to determine 1/n. This is the reason that no mean value of 1/n is given in each group. However, if the cases which showed low C'H50 values are omitted, the mean value and standard deviation of 1/n in the various disease groups are as follows: leukemias, 0.248 and 0.060, liver diseases, 0.229 and 0.044, collagen diseases, 0.258 and 0.048, kidney diseases 0.241 and 0.052. In general, sera with C'H50 values below normal tended to show high 1/n values. This may be one reason why above normal 1/n values were obtained in disease groups. However, the differences in the 1/n values in various disease groups should be investigated further.

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