

Serum Levels of β_{1C} Globulin, a Complement Component, in the Nephritides, Lipoid Nephrosis, and Other Conditions *

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Evidence for the participation of complement in the immune reaction responsible for renal lesions in the nephritides was first reported by Gunn in 1915 (1), who showed that complement was reduced in the serum of patients with acute nephritis following scarlet fever. Subsequently, reduced serum complement levels have been reported not only in acute nephritis but also in subacute and chronic nephritis, disseminated lupus erythematosus with renal involvement, lipoid nephrosis of children (2-5), and in the nephritis resulting from experimental hypersensitivity in animals (6).

The recent isolation and identification of various complement components as discrete proteins allow a new approach to the study of complement participation in renal disease. One of these components, known from its position in the immunoelectrophoretic pattern as β_{1C} -globulin, has been studied extensively by Müller-Eberhard, Nilsson, and Aronsson (7) and Müller-Eberhard and Nilsson (8). These workers have found, from immunoelectrophoretic analyses of serum, that this protein can be present in two forms: β_{1C} and β_{1A} . β_{1C} is present only in fresh serum; as the serum ages, the arc representing β_{1C} is replaced by one representing β_{1A} , a protein of slightly greater mobility. In serums of intermediate age, both β_{1C} and β_{1A} can be seen, the arcs joining to produce a single arc with a double inflection. Conversion of β_{1C} to β_{1A} can be hastened by incubating the serum at 37° C or by adding zymosan, hydrazine, im-

mune precipitates, or aggregated gamma globulin to the serum. It has been demonstrated that these two proteins have antigens in common, but that β_{1A} lacks certain antigens possessed by β_{1C} . The lability of β_{1C} led to its investigation as a part of the complement complex. It was found that β_{1C} has serological activities in a hemolytic system similar to those of the third component of complement, but that it does not represent the whole of the third component. B_{1A} was found to be serologically inactive. Further studies (9) have identified β_{1C} with the hydrazine-sensitive moiety of C_3 known as C'_{3a} .

Evidence suggesting that this globulin is involved in renal disease was first presented by Seligmann and Hanau in 1958 (10). They reported reduced levels or absence of a β -globulin in the serum immunoelectrophoretic patterns of patients with disseminated lupus erythematosus with renal involvement. With clinical improvement, the protein, which was undoubtedly β_{1C} , returned to its normal concentration. Morse, Müller-Eberhard, and Kunkel (11) have recently confirmed the absence of β_{1C} -globulin in this disease. Equally important, studies with the immunofluorescent technique have indicated the presence of β_{1C} -globulin in high concentration in the glomerular lesions in lupus nephritis (12).

The present study was prompted by the observation that, by immunoelectrophoretic analysis, β_{1C} -globulin is in low concentration not only in disseminated lupus but also in the serum of patients with acute nephritis. This observation stimulated the development of a method for the measurement of this protein, using the principle of an immunologic method described previously for gamma $_{1A}$ and gamma $_{1M}$ globulins (13). Results are reported of measurements made on the serums of patients with acute and chronic nephri-

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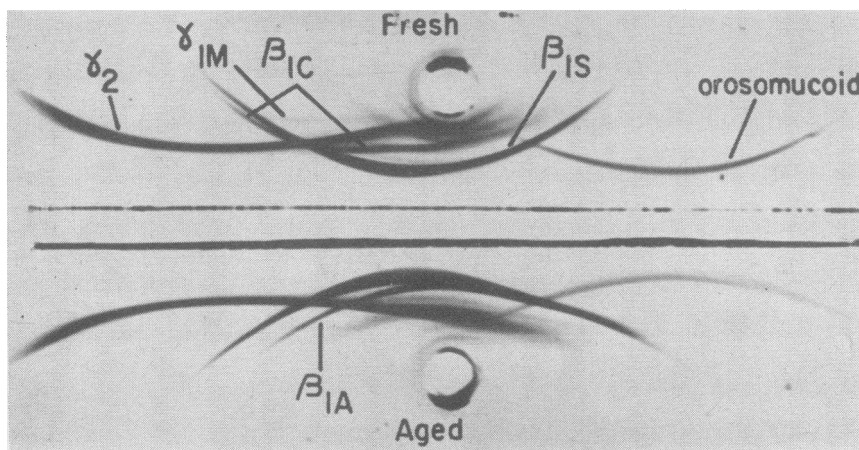


FIG. 1. IMMUNOELECTROPHORETIC PATTERNS OF FRESH AND AGED SERUM.

tis, lipid nephrosis of childhood, and anaphylactoid purpura with nephritis. Also included are the values in a few patients with chronic pyelonephritis, idiopathic familial hematuria, and severe liver disease and in one patient with lupus nephritis. The results give clear-cut differences among the various types of renal disease in the involvement of this complement component. In certain types, the levels are markedly reduced, whereas in others they are normal. Because serum β_{1C} depletion in renal disease is most likely the result of fixation of this protein with other complement components on immune complexes in renal glomeruli, the measurement of serum β_{1C} levels provides an additional parameter helpful in determining the basic etiology of nephritis. Serum β_{1C} levels can be measured more precisely than can serum complement activity and, in several ways, give information of greater value.

Methods

β_{1C} - β_{1A} -globulin concentration was measured in arbitrary units by the immunoelectrophoretic-precipitin method (13) with an extended schedule of titrations to permit assessment of very low values (14).

Antiserum. The antiserum used for the measurement was made in goats by immunization with a series of injections of whole human serum in Freund's adjuvant. Antibody to β_{1C} - β_{1A} was present in relatively high titer along with antibody to numerous other serum proteins. The antiserum was diluted until addition of 0.030 ml of a pool of normal human serum per milliliter of diluted antiserum completely removed the antibody reacting with β_{1A} -globulin. A dilution of 1:8 was required.

β_{1A} -enriched preparation. A euglobulin precipitate was prepared from 440 ml of normal human serum that had

been allowed to age to convert β_{1C} to β_{1A} . The precipitate was prepared according to the first step in the procedure for the isolation of β_{1A} as described by Müller-Eberhard and associates (7). A stock solution was made of the precipitate by dissolving in 10 ml of Tris-hydrochloride buffer, 0.5 N, pH 8.6, and centrifuging to remove the lipids. The stock solution has been unaffected by storage for 1 year at -10°C .

To calibrate the immunoelectrophoretic-precipitin method for β_{1A} , 1 ml of the antiserum, diluted 1:8, was arbitrarily said to contain antibody equivalent to 1 U of β_{1A} -globulin. From this, the concentration of β_{1A} -globulin (S) in an unknown serum expressed in units per milliliter could be determined by the formula $S = A/V$, in which A is the units of β_{1A} precipitated by antibody in 1 ml of the standardized antiserum (arbitrarily assumed, in this case, as 1 U), and V the volume of unknown serum added per milliliter of standardized antiserum which resulted in an end point. In the case of the pool of normal human serum noted above, the addition of 0.03 ml to 1 ml of the diluted antiserum resulted in an end point. Thus the concentration of β_{1A} in the pool was 33.3 U per ml.

To determine the end point, the supernatant fluids resulting from the graded additions of serum to the standard antiserum were reacted against the β_{1A} -enriched preparation, diluted 1:64, and subjected to electrophoresis in agar by the microtechnique of Scheidegger as described previously (13). At the dilution of 1:64, the β_{1A} concentration in the agar was such that supernatant fluids near the end point containing antibody to β_{1A} in low concentration produced a well-defined precipitin arc representing this protein. With certain supernatant fluids, other arcs could also be seen, but with practice, β_{1A} could be readily distinguished from them.

Measurement of the concentration of this β -globulin in serum specimens when fresh and after aging showed that consumption of antibody reacting with β_{1A} increased with aging. For example, 1 ml of standard antiserum could be depleted of anti- β_{1A} by addition of 0.045 ml of

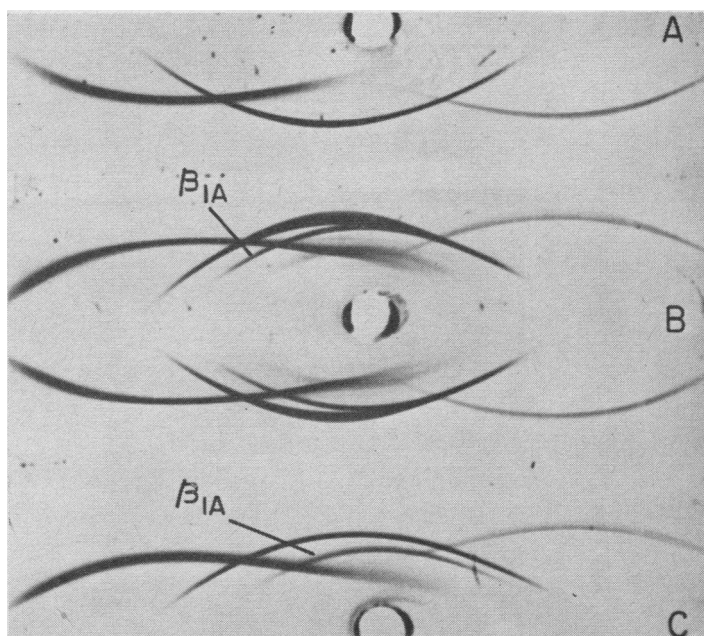


FIG. 2. COMPARISON OF IMMUNOELECTROPHORETIC PATTERN OF NORMAL SERUM (B) AND SERUM FROM PATIENTS WITH NEPHRITIS (A AND C). The antiserum is the same as in Figure 1. All serum has been aged. In A, the β_{1A} level is 1.6 U per ml; in the normal serum B, the level is 30 U per ml, and in C it is 11 U per ml.

a fresh serum, but only 0.025 ml was required after the serum aged and conversion to β_{1A} had occurred. It was thus essential that measurements be consistently made when the protein was either in the β_{1C} or β_{1A} form. Because stored serum was often used in the present study, measurements were always made when the protein was in the β_{1A} form, and the results are expressed in terms of β_{1A} . The conversion to β_{1A} was brought about by aging the serum, either for 72 hours or more at room temperature, or for 8 hours at room temperature and a week to a year at -10°C . Separate studies indicated that these periods allowed conversion to occur without resulting in loss of β_{1A} through bacterial contamination; specimens left at room temperature for 2 weeks showed no reduction in the β_{1A} level from the peak value.

Results

In Figure 1 are illustrated immunoelectrophoretic patterns of fresh and aged serum that allow comparison of the relative electrophoretic mobility of β_{1C} - and β_{1A} -globulins. Precipitin arcs representing other proteins are labeled for orientation. In fresh normal serum, β_{1C} -globulin is the only form seen and β_{1A} -globulin is absent. However, in the fresh serum from patients with lupus, the β_{1A} form of the globulin has been reported to be pres-

ent (11, 15). In the present study, β_{1A} was not seen in fresh serum.

Figure 2 compares the β_{1A} arc seen in aged normal serum (B) with that produced by serums deficient in β_{1A} (A and C). In the upper pattern, the β_{1A} concentration is so low that an arc is not visible.

Serum β_{1A} levels in normal subjects. The results of quantitative measurement of β_{1A} concentration in the serums of 10 normal adults and 45 normal children and adolescents, ages 8 to 18 years, are shown in the upper portion of Figure 3. There was no significant difference between these two age groups nor was there a difference between males and females. In general, the values ranged between 25 and 45 U per ml, but one value as high as 50 and another as low as 17 were observed. The average of all normals was 33.4 U per ml. A specimen of pooled serum from cord blood gave a value of 23 U per ml.

From these results and from observations in this laboratory of many immunoelectrophoretic patterns that allow a rough semiquantitative estimate of β_{1C} - β_{1A} concentration, it appears that the

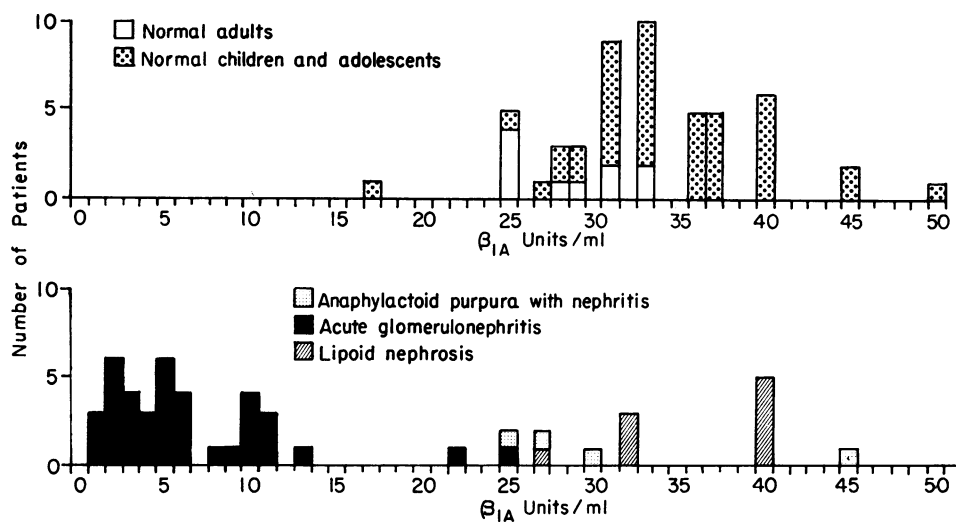


FIG. 3. SERUM β_{1A} LEVELS IN NORMAL SUBJECTS (upper graph) AND IN CHILDREN WITH ANAPHYLACTOID PURPURA WITH NEPHRITIS, ACUTE NEPHRITIS, AND LIPOID NEPHROSIS (lower graph).

serum content of this protein does not differ in children and in adults.

Serum β_{1A} levels in the nephritis of anaphylactoid purpura and lipoid nephrosis. In the lower portion of Figure 3 are shown the results of β_{1A} measurements in the serums of nine children with lipoid nephrosis, four with anaphylactoid purpura with nephritis, and 37 with acute nephritis. The nephrotics ranged in age from 1½ to 15 years. All had a disease characterized by remissions and exacerbations and had no evidence of renal insufficiency or nephritis. Serum specimens were obtained at a time when proteinuria was intense and the patient was not receiving steroids. It is readily apparent that levels of β_{1A} in this group fall within the normal range.

Similarly, levels of this globulin were normal in patients with the nephritis of anaphylactoid purpura. These patients ranged in age from 3 to 9 years. In three of the children, the purpura and urinary abnormalities were concomitant, and blood for β_{1A} measurement was obtained within 2 to 6 days after onset of the purpura. In one child, urinary abnormality did not occur until 4 weeks after the rash had faded. In all cases, hematuria or proteinuria, or both, were present at the time blood for β_{1A} measurement was drawn. None of the children is known to have permanent renal damage as a result of the disease.

Acute nephritis. In contrast to the results in

anaphylactoid purpura and nephrosis, children with acute nephritis, with two exceptions, had β_{1A} levels below the normal range. The children in the acute nephritis group were 1½ to 15 years of age. All had well-documented hematuria and proteinuria and, usually, in addition, edema, hypertension, and azotemia in various combinations. Antistreptolysin O titer, determined in 35 of the 37 patients at least once in the 3-week period after admission, was above 150 U in 31, or 89%. This percentage is approximately the same incidence of elevation as observed by Lyttle, Seegal, Loeb, and Jost in a larger group (16). In all 37 patients except one of the two with a normal β_{1A} level, the disease was self-limited and short-lived with all signs of renal disease eventually disappearing. Biopsies were performed on only two patients; both showed acute nephritis. The blood specimens for β_{1A} determination were obtained usually during the first 3 days after admission, but a few were obtained as late as 7 days after admission. No correlation between duration of the disease and β_{1A} levels was apparent, even when the period of illness before admission was taken into consideration.

Of the two patients with relatively normal levels of β_{1A} , one was a 3-year-old boy who appeared to have a mild, transient acute nephritis, and no reason for the normal β_{1A} level can be given. He was admitted because of hematuria occurring for

the first time on the day of admission; proteinuria was also present, but there was no edema, hypertension, or azotemia. Antistreptolysin titer was 500 U. The proteinuria disappeared by the second hospital day, but microscopic hematuria persisted for several days and eventually cleared also.

The other patient with a normal β_{1A} level was a 10-year-old boy on whom a urinalysis was prompted on the day of admission by the complaint of backache and abdominal pain. Proteinuria and hematuria were noted. A sore throat had been present 3 weeks previously and was treated with penicillin. The blood urea nitrogen (BUN) was elevated to 37 mg per 100 ml, but cholesterol and total serum protein levels were in the normal range. Antistreptolysin titer was 333 U. Measurement of β_{1A} on the day of admission and 5 days and 1 month after admission gave values ranging from 22.2 to 33.3 U per ml. There was no hypertension and edema was not apparent. The BUN fell to normal promptly, and 2 weeks after admission, urea clearance was 75% of normal. However, proteinuria persisted at the 2+ to 4+ level for 6 months, subsequently falling to the 0 to 1+ range. Red blood cells, however, have persisted in the urine in microscopic amounts for 11 months after onset. Renal biopsy done 3 weeks after admission indicated an acute proliferative glomerulonephritis to be present. The glomeruli were focally hypercellular, but there was no sign of subacute or chronic disease. A second biopsy

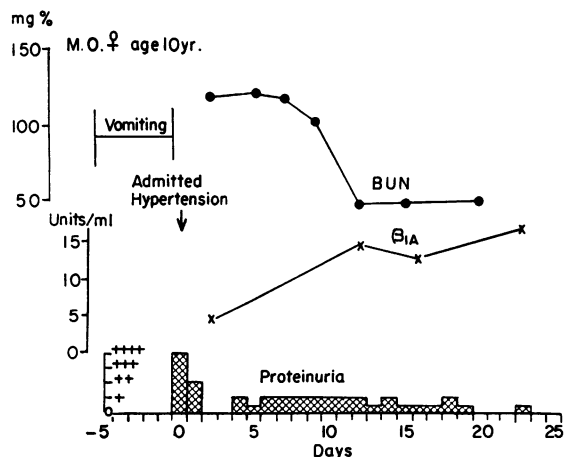


FIG. 4. CLINICAL COURSE, SERUM β_{1A} LEVELS, PROTEINURIA, AND BLOOD UREA NITROGEN (BUN) IN A PATIENT WITH ACUTE NEPHRITIS.

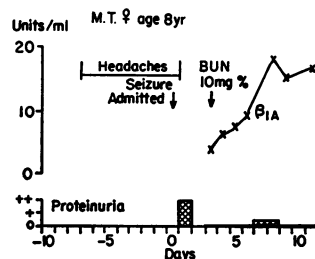


FIG. 5. CLINICAL COURSE, SERUM β_{1A} LEVELS, AND PROTEINURIA IN A PATIENT WITH ACUTE NEPHRITIS.

10 months after onset showed almost complete healing without residual. A mild hypercellularity was still present, but there was no basement membrane thickening or scarring. The persistence of urinary abnormalities in this boy, together with the consistently normal β_{1C} level, would suggest that he had a disease outwardly similar to, but fundamentally different from, classical acute nephritis. It may possibly be etiologically similar to the chronic nephritis that is accompanied by normal β_{1C} levels (see below).

In patients with acute nephritis and reduced β_{1A} levels, the levels rose to normal in parallel with clinical improvement. The results of serial measurements are illustrated for three patients in Figures 4, 5, and 6. M. O. (Figure 4) had a course characterized by hypertension, severe and persistent azotemia, and persistent proteinuria. In this patient, the level of β_{1A} rose slowly, apparently reaching the normal range before the BUN returned to normal.

M. T. (Figure 5) probably had hypertension for a week before her admission although hypertension was never documented. Seizures, together with a low-grade fever on admission, suggested encephalitis, meningitis, or brain tumor. The symptoms of abnormality of the central nervous system persisted, and a mild, transient proteinuria and hematuria were overlooked until 36 hours after admission when the additional history of antecedent sore throat prompted the diagnosis of acute nephritis. In favor of this diagnosis was the observation of absence of β_{1C} -globulin by immunoelectrophoretic analysis (actual level of β_{1A} was 2 U per ml). On subsequent days, her symptoms and urinary abnormalities cleared rapidly and, similarly, the level of β_{1A} rose rapidly to normal.

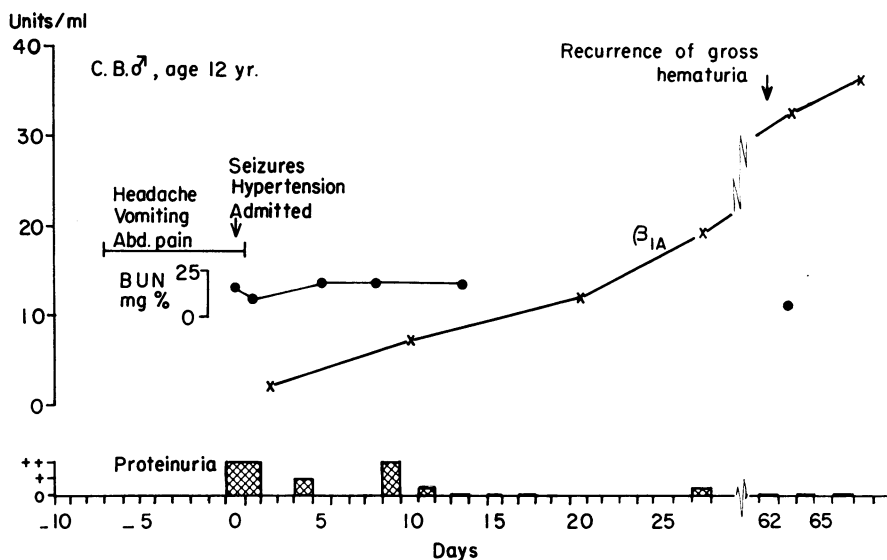


FIG. 6. CLINICAL COURSE, SERUM β_{1A} LEVELS, PROTEINURIA, AND BUN IN A PATIENT WITH ACUTE NEPHRITIS. Recurrence of gross hematuria at 62 days after initial admission apparently occurred spontaneously without provocative infection.

C. B. (Figure 6), whose nephritis followed scarlet fever, likewise had hypertension and seizures and, similar to M. T., had no azotemia. Improvement in urinary abnormalities and rise in β_{1A} occurred slowly. On day 62 after the seizures he was found to have a recurrence of severe hematuria, but he had no proteinuria, hypertension, azotemia, edema, or intercurrent infection. Despite the relapse, the β_{1A} level remained in the normal range.

Two other patients had apparent relapses of their nephritis while under observation, one as the result of an otitis media and the other concomitant with pneumonia. As with C. B., the chief event in the relapse was increased hematuria without proteinuria, azotemia, or hypertension. Although both were said to have facial edema, neither showed a weight gain or loss suggesting generalized edema. In neither was the β_{1A} level, which had reached normal, affected by the relapse.

TABLE I
Serum β_{1A} levels in chronic nephritis

Patient	Sex	Age at onset	Duration of disease	β_{1A} Levels		Number of β_{1A} determinations	Period β_{1A} levels obtained*
				Average	Range		
		yrs, mos	months		U/ml		months
P. B.	F	2, 10	11	31	(26-42)	5	7
R. L. W.	M	5	19	1.7	(1.3-2.3)	11	19
R. W.	M	6, 11	54	3.7	(2.0-5.7)	10	32
S. S.	M	7, 6	13	5.3	(1.7-13.3)†	27	12
L. B.	F	9, 1	73	1.8	(1.7-2.0)	2	18
G. C.	M	9, 6	77	8.7	(2.0-16.1)†	23	39
J. T.	F	11, 3	23	38	(31-40)	6	22
L. S.	M	12, 1	20	30	(20-40)	6	19
R. S.	M	12, 1	8	7.2	(1.7-15.4)†	15	8
B. H.	F	12, 2	9	30	(25-44)	8	9
V. C.	F	13, 1	18	26	(22-31)	4	6
D. C.	M	14, 4	9	36	(31-44)	5	7

* Length of period in course of the nephritis during which the serum β_{1A} levels were determined.

† Values above 11 U per ml obtained during or shortly after use of immune-suppressing therapy.

TABLE II
Serum β_{1A} levels in lupus erythematosus, chronic pyelonephritis, hereditary hematuria, and miscellaneous diseases with liver involvement

Patient	Sex	Age	β_{1A} levels		Number of β_{1A} determinations	Remarks
			Average	Range		
		<i>yrs</i>		<i>U/ml</i>		
Disseminated lupus erythematosus						
T. S.	F	9	4.5	(4-5)	6	Severe lupus nephritis. Blood urea nitrogen 75-120. Proteinuria, hematuria, hypertension, leukopenia, hepatosplenomegaly. No rash.
M. S.	M	13	21.7	(20-23.6)	4	No nephritis. Splenomegaly, diffuse adenopathy, arthritis. On Dilantin and Tridione for long period.
Chronic pyelonephritis						
J. F.	F	10	22.2		1	Severe hypertension and azotemia. Death 5 days after specimen.
R. S.	F	13	27.2	(21.1-33.3)	2	Azotemia, acidosis, renal osteodystrophy.
Idiopathic hematuria						
L. F.	F	5	44		1	Mother and two children. Constant microscopic hematuria. In L.F., gross hematuria with infection. No renal insufficiency.
M. F.	M	7	36		1	
B. F.	F	27	33		1	
R. H.	M	10	36		1	Mother and son. Microscopic hematuria. No renal insufficiency.
G. H.	F	34	33		1	
Other conditions with low β_{1C} levels						
T. H.	F	6	3.3		1	Acute lymphoblastic leukemia for 4 months. Severe hepatosplenomegaly, icterus. Death 2 days after determination.
D. F.	M	15	9		1	Hereditary spherocytosis with severe hepatosplenomegaly. Total bilirubin, 3 mg per 100 ml. Thymol and cephalin flocculation, 4+.
D. R.	M	11	14.3		1	Cirrhosis, hypoprothrombinemia, varices, mental retardation.
W. G.	M	15	6.6		1	Cirrhosis with splenomegaly, thrombocytopenia, leukopenia, hypoprothrombinemia, mental retardation.
T. V. C.	F	14	6.2		1	Acute diffuse hepatic necrosis due to viral hepatitis. Hepatic coma. Complete recovery.

Chronic nephritis. In Table I are shown β_{1A} levels in 12 patients with long-standing glomerulonephritis. The levels were determined over intervals of varying lengths. All the patients had proteinuria for periods of 8 months or longer, accompanied in nearly every case by hematuria. All had biopsies that indicated glomerulonephritis to be present in acute, subacute, or chronic form. None had demonstrable lupus erythematosus cells or antinuclear antibody, and there were no clinical signs compatible with lupus except the nephritis.

Beta_{1A} levels in six of these patients were consistently in the normal range and in the other

six were consistently below the normal range. Those with normal levels are part of a larger group totaling 15 patients, whereas only the six shown here have been found with depressed levels. Superficial examination of the clinical courses and biopsies of these patients showed no clear-cut difference among them which correlated with the β_{1C} level. A detailed comparison of the two groups will be reported in a subsequent publication.

Lupus erythematosus, chronic pyelonephritis, hereditary hematuria, and diseases with liver involvement. Data are available from only two pa-

tients with lupus erythematosus. One, T. S., with lupus nephritis, had a reduced β_{1C} level, whereas the other, M. S., without signs of nephritis, had a normal level. In patient T. S., the levels came to normal with steroid therapy, paralleling clinical improvement.

Patients with pyelonephritis and hereditary hematuria (17) had uniformly normal levels.

Data for several patients with disease not involving the kidneys but with depressed β_{1C} levels, ranging from 3.3 to 14.3 U per ml, are also shown in Table II. The primary diseases in these patients were lymphoblastic leukemia, hereditary spherocytosis, cirrhosis, and hepatic necrosis. The common denominator in all was liver involvement. In three patients, there was obvious cirrhosis or necrosis, and in two, the liver was secondarily involved as indicated by hepatomegaly, positive liver flocculation tests, and jaundice. In patients with milder degrees of liver disease such as acute hepatitis, the β_{1C} levels were invariably normal.

Semiquantitative estimations of β_{1C} levels have been made from immunoelectrophoretic analyses of the serum from numerous patients with a wide variety of diseases. From this experience, we can say that in addition to liver disease and the nephritides, low levels can also be seen occasionally in moribund patients with neither liver nor kidney disease. This perhaps parallels the depression in total complement seen under these conditions. No other disease states have been encountered in the pediatric age group in which the level is low.

Discussion

The reduction in serum β_{1C} -globulin concentration occurring in certain types of renal disease seems best ascribed to fixation of this protein, together with other complement components, on antigen-antibody complexes in the glomerular lesions. Although this explanation has long been advanced for the hypocomplementemia seen in renal disease, there has only recently been positive evidence for such a fixation.

Burkholder (18) demonstrated that the glomerular lesions of patients with membranous nephritis would fix guinea pig complement *in vitro*, suggesting that homologous complement might similarly fix *in vivo*. More directly, Lachmann, Müller-Eberhard, Kunkel, and Paronetto

(12) demonstrated that fluorescein-labeled antibody to β_{1C} -globulin would fix abundantly on the glomerular lesions of patients with lupus and less abundantly on the lesions of acute nephritis. The less distinct evidence for β_{1C} fixation in acute nephritis was probably because the biopsy material was in no case obtained at the height of the disease. The present studies of serum β_{1C} levels give evidence that fixation of this protein in the renal lesions of acute nephritis is a transient event and would be demonstrable in many cases only for a matter of days.

Lachmann and associates (12) also showed that at sites of β_{1C} fixation in the glomeruli in lupus nephritis, gamma₂ globulin could also be demonstrated, presumably present either in aggregated form or, more likely, as part of an antigen-antibody complex. That β_{1C} as a complement component could fix to such complexes has been indicated by an *in vitro* system which demonstrated (12) that, when antibody reacts with tissue antigens, fixation of β_{1C} occurs under the same conditions as when this protein participates in immune hemolysis, i.e., its fixation in both systems is temperature-dependent and requires the presence of other complement components.

Further evidence of *in vivo* complement fixation in nephritis comes from the observation that in certain cases of lupus nephritis β_{1A} -globulin can be found in fresh serum (11, 15). Since β_{1C} is converted to β_{1A} by complement-fixing material such as immune precipitates and aggregated gamma globulin, the observation is strongly suggestive of *in vivo* complement fixation.

Despite these observations, however, direct and unequivocal proof that fixation of β_{1C} as a complement component is entirely responsible for the reduced serum levels in renal disease is not at hand. Other possibilities are excessive urinary excretion, failure of production, or nonspecific fixation of β_{1C} in the renal lesions. Loss of β_{1C} -globulin by urinary excretion, a suggested but unproven (19) explanation for the low complement levels in nephritis, would seem not to be the factor responsible for the low serum levels of β_{1C} . Proteinuria comparable to or more severe than that present in acute nephritis was seen in patients with nephrosis and in most of those with anaphylactoid purpura, yet these patients had normal β_{1C}

levels. Proteinuria in patients with chronic nephritis with normal β_{1C} levels was often more severe than that in patients with low levels. A differential permeability for β_{1C} in the kidney of patients with acute nephritis and certain patients with chronic nephritis would have to be postulated to explain the loss of β_{1C} by renal excretion. There is no evidence for or against failure of production as a cause of the low serum levels, but such a mechanism, as well as nonspecific fixation in renal lesions, seems unlikely in the face of the reduction of other complement components which occurs simultaneously.

The results of β_{1C} measurement as given in the present study parallel the measurements by others of total serum complement in all situations except in lipid nephrosis. In the latter condition, serum complement has been found to be low (2), but β_{1C} is normal. The complement components found to be reduced in nephrosis are C'_4 and, more consistently, C'_2 . If the reduction in these two components were the result of fixation to an immune complex, the complex $C'_{0,1,4,2}$ should be present. Since β_{1C} is the next component in this sequential reaction, one would predict a reduction in its serum level. The fact that the level is normal suggests that the reduction in C'_2 and C'_4 is not the result of immune fixation and that other mechanisms are responsible for the hypocomplementemia in lipid nephrosis.

Except for this discrepancy, complement and β_{1C} levels, appear, in general, to parallel each other. Thus, in acute (1-4) and lupus nephritis (4, 11) and in occasional cases of chronic nephritis (3, 5), complement levels are low, whereas in the nephritis of anaphylactoid purpura the levels are normal (4). Likewise, the low β_{1C} levels in liver disease agree with earlier observations that serum complement is depressed in this condition (15). An explanation of the reduced levels in liver disease is not at hand. To explain the reduction on the basis of failure of hepatic synthesis of β_{1C} is difficult because of evidence that β_{1C} is produced in lymph nodes and spleen (20). Alternatively, the low levels might be the result of a complement-binding reaction of liver antigen with autoantibody.

Separation of two groups of patients with chronic nephritis on the basis of β_{1C} levels is of

interest because of the likelihood that the classification reflects differing etiologies. In those with low levels, the disease would seem most likely to be on the basis of a reaction of humoral antikidney antibody with glomerular tissue, whereas in those with normal levels a different etiology must be invoked. In another paper, the two groups will be compared to determine additional points of difference based on clinical and histological parameters. Suffice it to say here that the β_{1C} levels in these patients were consistently either low or normal over long periods. This is in contrast to the studies of serum complement in subacute nephritis and "uncomplicated nephrotic syndrome" by Ellis and Walton (5). These authors report that in subacute nephritis serum complement is close to the lower limit of normal, whereas in "uncomplicated nephrotic syndrome" two groups seemed distinguishable, one with subnormal and the other with normal complement levels. However, the separation into two groups was not entirely satisfactory because, in contrast to the observations with β_{1C} , the levels of complement were not consistent, often rising as the patient improved or as his condition deteriorated.

Semiquantitative assessment of β_{1C} -globulin would appear to be a helpful clinical parameter. The majority of patients with near absence or low levels of β_{1C} -globulin would be those with acute nephritis, a disease at present diagnosed only by a spectrum of abnormalities, no one of which is consistently present (21). Low levels would also be seen in cases of lupus nephritis and in a minority of cases of chronic nephritis. Normal levels in a subject with an apparent acute nephritis would appear to be reason to investigate for signs of chronic nephritis or to suspect hereditary hematuria or nephritis of anaphylactoid purpura. Patients with Alport's disease or with polyarteritis nodosa have not been investigated with respect to β_{1C} levels.

Of particular interest is the patient observed in the present study with normal β_{1C} levels and an acute nephritis that proved to be particularly persistent. One might ask whether this patient's disease is etiologically similar to that of patients with chronic nephritis and normal β_{1C} levels. At the moment, one can only speculate on the answer to this question, but as more such cases are identified

and followed, our understanding of the etiology of chronic nephritis may be improved.

The immunoelectrophoretic-precipitin method for measurement of β_{1C} used in the present study gives highly accurate results but is somewhat cumbersome and time consuming to perform. It is, however, less difficult than the measurement of total complement, and the results are more reproducible. Because it is a more reliable measurement and, in addition, measures only the concentrations of a single substance instead of the effect of the sequential reaction of many complement components, the results are inherently more meaningful. Furthermore, anticomplementary factors and lipids in serum do not interfere with the β_{1C} measurement. For clinical purposes, much information can be gained by the rough estimate of β_{1C} concentration possible from the immunoelectrophoretic pattern with a suitable antiserum (Figure 2). With the availability of a univalent antiserum containing antibody only to this protein, a simpler, more accurate and rapid method for measurement probably could be developed, based either on the method of Gell as elaborated by Soothill (22) or that of Heiskell and associates (23).

Summary

By an immunologic method, measurements of β_{1A} -globulin, the serologically inactive form of β_{1C} -globulin, have been made in serum specimens from normal subjects and from children with various nephritides, nephrosis, and diseases in which the liver was involved.

In 35 of 37 children with acute nephritis, β_{1A} -globulin was nearly absent from the serum or in very low concentration. In one of the two children with normal levels, the nephritis was unusually persistent, suggesting that the disease was not a classical acute nephritis. In the other, the normal level is unexplained. Serial measurement of β_{1A} -globulin in three patients with acute nephritis and depressed levels showed a return to normal over a period of approximately 3 to 6 weeks paralleling clinical improvement. Exacerbations of hematuria occurring during convalescence from acute nephritis, often associated with intercurrent infection, were not accompanied by a fall in β_{1A} levels.

In lipid nephrosis and in the nephritis of anaphylactoid purpura, β_{1A} -globulin was in normal concentration.

Beta_{1A} levels were low in a case of lupus nephritis as well as in a minority of cases of chronic nephritis. The latter patients were not easily distinguishable clinically from patients with chronic nephritis and normal β_{1C} levels. Normal levels were present in hereditary hematuria and in chronic pyelonephritis and in one patient with lupus but without evidence of nephritis. Low levels were encountered in five patients with severe liver disease. In three the liver disease was primary, in one, secondary to lymphoblastic leukemia, and in another, secondary to hereditary spherocytosis.

The reduction of β_{1C} -globulin in the various types of nephritis is probably the result of fixation of this protein together with other components of complement to immune complexes in the glomerular lesions. In lipid nephrosis, in the nephritis of anaphylactoid purpura, and in the majority of cases of chronic nephritis, the immune reaction, if it exists, must have different characteristics with regard to complement fixation.

Quantitative assessment of β_{1C} - β_{1A} -globulin would appear to have clinical value in the differentiation of various types of nephritis.

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Addendum

Since the preparation of this manuscript, the β_{1A} protein equivalence of the arbitrary units in which the immunoelectrophoretic-precipitin method was calibrated has been determined. Two preparations of purified β_{1A} -globulin were made from aged serum by one of us (N. C. D.) employing a modification of the method of Müller-Eberhard and associates (7). The first preparation, with a β_{1A} concentration of 54 U per ml showed, by immunoelectrophoretic analysis with antisera from goats highly immunized to whole human serum, a faint, fuzzy precipitate in the β region, in addition to a sharp β_{1A} arc. Replicate determinations of nitrogen by the micro-Kjeldahl technique and of β_{1A} concentration by the immunoelectrophoretic-precipitin method gave results indicating

that 1 U was equal to 51 μ g of protein. The second preparation, in a concentration of 18.4 U of β_{1A} per ml, showed no contamination with other proteins by immunoelectrophoretic analysis. In this preparation, 1 U was found to be equivalent to 45.7 μ g of protein. In normal subjects the mean β_{1A} concentration of 33 U per ml would thus be equivalent to 151 mg per 100 ml.

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